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Improving Growth and Survival of Cultured Yellow Lampmussel (Lampsilis cariosa) for Restoring Populations

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IMPROVING GROWTH AND SURVIVAL OF CULTURED YELLOW LAMPMUSSEL *(LAMPSILIS CARIOSA)* FOR RESTORING POPULATIONS

A Thesis Presented

by

VIRGINIA MAE MARTELL

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DEDICATION

To my husband:

for his patience, encouragement, and for being the best cheerleader I could have had

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ABSTRACT
IMPROVING GROWTH AND SURVIVAL OF CULTURED YELLOW LAMPMUSSEL *(LAMPSILIS CARIOSA)* FOR RESTORING POPULATIONS

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In North America 72% of freshwater mussel species are endangered, threatened, or of special concern due to factors such as habitat loss and degradation, biological invasion, and land use change. Propagation of freshwater mussels has been considered a necessary conservation strategy for population restoration where threats have been mitigated but small population sizes limit population viability. Yellow lampmussel is a species of freshwater mussel that is endangered, threatened, or imperiled throughout its range; therefore, I evaluated laboratory techniques (probiotic supplements and secondary rearing designs) to improve culture of yellow lampmussel for population restoration. Several aquaculture facilities commonly use probiotics; thus, I used commercial probiotics to determine if 1) probiotic concentration and 2) type of probiotic mixture improved growth or survival of juvenile mussels during primary culture. I further asked whether probiotics affected mussels by reducing ammonia, thereby improving water quality. Some probiotics increased survival (and, in one experiment, increased growth) of juvenile mussels, regardless of concentration, but results were variable by experiment and probiotic type. Probiotics did not significantly reduce ammonia concentrations, so this was unlikely the mechanism of benefit. I also investigated the effect of different

secondary rearing systems at two culture facilities on growth and survival of juvenile mussels in two size classes (<5.0 mm and >5.0 mm). I used five different secondary culture systems that were either indoors (dogpans and baskets) or outdoors (trough, airlift upweller, tank upweller, baskets), where water was either recirculating or flow-through. Survival was exceptional in all larger size class rearing systems, and the baskets in the ponds had the greatest growth rates. Smaller mussels had lower survival than the larger mussels, indicating that when deploying juvenile mussels into outdoor culture systems mussels size should be greater than 5.0 mm. Results of this project will inform future rearing yellow lampmussel in New England and more broadly add to the limited literature on probiotic use and secondary rearing designs in freshwater mussel culture.

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CHAPTER 1
INTRODUCTION

1.1 Freshwater Mussel Diversity, Distribution, and Ecosystem Services

Pearly freshwater mussels (herein termed "mussels") of the family Unionidae, inhabit all continents on the planet, except Antarctica. There are over 300 species within the family, with the majority (>250) found in the Nearctic region (Lopes-Lima et al. 2017). Mussels are key components of many freshwater ecosystems comprising a large proportion of benthic biomass and serving as ecosystem engineers in both lentic and lotic systems (Strayer et al. 1999, 2004). As sessile filter-feeders they remove phytoplankton, bacteria, and organic matter (Cohen et al. 1984) and strongly influence the nutrient cycle through burrowing, biodeposition, and nutrient excretion (Nalepa et al. 1991, Raikow and Hamilton 2000, Vaughn and Hakencamp 2001). Burrowing behavior oxygenates the sediment increasing microbial activity (e.g. denitrification); and mixes nutrients into the sediment while removing microbial wastes (McCall et al 1979, Hoellein et al. 2017). Biodeposition of feces and pseudofeces serve as food and nutrient sources for benthic invertebrates, and excretion of nitrogen and phosphorous may be directly used by phytoplankton and benthic algae (Vaughn and Hakencamp 2001). Mussels are also habitat modifiers, increasing benthic invertebrate diversity and abundance (Sephton 1980, Becket et al. 1996, Gutierrez et al. 2003). Mussels are a food source for molluscivorous fish (Roe et al. 1997, Magoulick and Lewis 2002), muskrats (Neves and Odom 1989), raccoon, otter, mink, and waterfowl. Because mussels serve a variety of ecosystem roles their activities can drastically alter aquatic ecosystems (synthesized by Vaughn and Hakencamp 2001, Vaughn et al. 2008, Vaughn 2018).

1. 2 Historical Declines and Conservation

Freshwater mussels are facing an unprecedented extinction crisis. In the last century, 10% of the North American native species have become extinct, and as of 2013, 74% of the remaining freshwater mussel species were considered imperiled (Williams 1993, Haag and Williams 2014). In early North America, freshwater mussels were a source of food, tool material, and ornaments for Native Americans (Parmalee and Klippel 1974, Christenson 1985), and early mussel population declines are thought to have been associated with increased agricultural activity of Native Americans (Peacock et al. 2005). Initial population declines of the late 19th and early 20th century have been attributed to the pearl and pearl button rush due to overharvesting and habitat degradation. Cultural use as decoration and jewelry persists today, although use has dramatically decreased (references therein Haag 2012). The meat of mussels has been used to make livestock feed, fertilizers, and fishery bait; and the shell has been used to produce limestone for products such as soaps and tiles (references therein Haag 2012). Population declines after the pearl button rush are likely due to widespread habitat loss and degradation (Vaughn et al. 1999, Haag 2009), biological invasion (Strayer 1999), eutrophication (Smith et al. 1999), land use change (Arbuckle and Downing 2002, Poole and Downing 2004, Peacock et al. 2005), and barriers to population movement such as host fish availability (Price et al. 2009). Without effective conservation programs, Ricciardi and Rassmusen (1999) predict the loss of at least 100 mussel species in the next century.

1. 3 Modern Conservation Efforts

Following the passage of the U.S. Endangered Species Act in 1973, 23 mussel species were listed as federally endangered. During the late 1970's, modern day pioneers in mussel

ecology, Richard J. Neves (Virginia Polytechnic and State University) and David L. Strayer (Cary Institute of Ecosystem Studies), began long-term research programs that included freshwater mussel biology, habitat, and sampling methods (Haag 2012). In 1993 the first assessment of freshwater mussels in North America was published (Williams et al. 1993), in 1997 the National Strategy for the Conservation of Native Freshwater Mussels was prepared by the National Native Mussel Conservation Committee (NNMCC 1998), and in 1998 the Freshwater Mollusk Conservation Society was formed (Lopes-Lima et al. 2014).

The National Strategy for Conservation of Native Freshwater Mussels set out ten goals and strategies for the conservation of freshwater mussels within two overarching themes. The biological theme included improving basic knowledge around freshwater mussel ecology, including habitat, population trends, threats, and potential methods of propagation and translocations of mussels. The second theme included dissemination of information, management actions, education, and funding (NNMCC 1998). With these goals in mind, research into freshwater mussel ecology flourished, with the 200 cumulative papers published by 1999 (Strayer et al. 2004) increasing to over 200 papers a year by 2014 (FMCS 2016). In the last three decades, knowledge of freshwater mussel ecology has broadened with private, academic, state, and federal agencies funding research and implementing freshwater mussel education and conservation programs. Today, there are at least 18 state and federal facilities researching various aspects of freshwater mussels for population restoration (Patterson et al. 2018), and at least 34 state agencies have a plan, or are developing a plan within the next 5 years, on freshwater mussel management (Bouska et al. 2018).

1. 4 Freshwater Mussel Propagation

Freshwater mussel propagation is an important tool for conservation and was first used to address the decline of economically important freshwater mussels around the peak (1910-1930) pearl and pearl button harvest. The first permanent laboratory station established to research ecology and techniques in propagation of freshwater opened in 1914, the U.S. Fisheries' Fairport Biological Station in Fairport, Iowa (Coker et al. 1921). The first *in-vivo* and *in-vitro* procedures were developed; however, because of limitations in finances, equipment, and effort, only stream-side inoculation and release of fish host were carried out. Although the Fairport Biological Station provided indispensable knowledge of freshwater mussel ecology, due to a lack of funding, interest, and limited success, the station was only operational from 1914–1933 (Pritchard 2001).

In 1998, nearly three-quarters of a century after the Fairport Biological Station closed, the first modern propagated mussels were released (Neves 2004). Studies in improvement of survival and growth of freshwater mussels under culture conditions has increased in the last two decades. Because mussels are highly vulnerable after metamorphosis, culturing practices of mussels is typically categorized by juvenile mussel size to improve survival and growth. Primary culturing of juvenile mussels consists of newly metamorphosed and young juvenile mussels up to 5 mm in length (or when growth rates slow) and secondary culture is considered larger (>3–5 mm) juvenile mussels (Patterson et al. 2018). Experimental studies in primary juvenile mussel have included temperature (Beaty and Neves 2004, Pandolfo et al. 2010, Carey et al. 2013), feed ratio and diet (Gatenby et al. 1996, 1997; Gatenby 2000; Henley et al. 2001, Beck 2001, Mair 2013, Hemchandra et al. 2015), bacterial supplementation (or 'probiotic' use) (Gatenby 2000, Vincie 2008), sediment inclusion

(Gatenby et al. 1996, Jones et al. 2005, Beaty and Neves 2004, Liberty et al. 2007), ammonia nitrogen build-up (Augspurger et al. 2003), and predator control (Zimmerman et al. 2003). These experiments have helped to standardize procedures for mussels at their most vulnerable life-stage. Many of the guiding principles from primary culture experiments (e.g. thermal tolerance, nutrition needs, etc.) transfer to secondary culture; however, the construction and design of culture systems can vary widely. The ability to change culture design provides several secondary rearing options that can be tailored to both facility and species needs. Secondary culture systems include: floating baskets (Mummert 2001), upwelling systems (Mair 2013), dogpans (Mair 2013), aquaria (Zimmerman 2003, Kotitvahdi et al. 2008), buckets (Barnhart et al. 2006, Mair 2013), sand trays (Yang 1996), troughs (Hanlon 2000, Mummert 2001, Zimmerman 2003), cages (Buddensiek 1995, Gatenby 2000, Brady et al. 2011), nets (Gatenby 2000), and bunkers which can be deployed in simulated stream channels, hatchery raceways, ponds, or as recirculating systems (Dunn and Layzer 1997, summary of secondary rearing systems in Patterson et al. 2018). Advancements in knowledge of mussel ecology and laboratory procedures, as well as increased availability of facility space, funding, and interest have provided a substantial boost the success of culturing practices of several unionid species. By 2010, over 2 million freshwater mussels had been released into restoration sites from at least 5 different facilities (Haag 2012, FMCS 2016). However, for several species physiological requirements and culture practices still need to be refined (FMCS 2016).

In Massachusetts, propagation of freshwater mussels has been identified as a potential option in freshwater mussel population restoration (MDFW 2015). Massachusetts has 12 native freshwater mussel species, of which 1 is federally endangered (*Alasmidonta*

heterodon), 2 additional species are state endangered (*Alasmidonta varicosa, Lampsilis cariosa),* and 3 are special concern (*Leptodea ochracea, Ligumia nasuta, Strophitus undulatus)*. Due to the status of freshwater mussels in Massachusetts, a multi-year cooperative partnership was established between the U.S. Fish and Wildlife Service (USFWS), the U.S. Geological Survey's Massachusetts Cooperative Fish and Wildlife Research Unit, and the Massachusetts Division of Fisheries and Wildlife. In 2015, under the cooperative partnership, the USFWS's Richard Cronin Aquatic Resource Center (CARC) in Sunderland, Massachusetts began research as the first mussel propagation facility in New England. One of the goals identified by the cooperative partnership was to research and inform conservation of the yellow lampmussel *(Lampsilis cariosa)*; as such, research into propagation and culturing of yellow lampmussel began in 2017.

1.5 Study Species – The Yellow Lampmussel (*Lampsilis cariosa)*

1.5.1 Morphology

Yellow lampmussel are medium-sized species that can reach a total length of 134 mm (Nedeau 2008). They have a moderately thick shell, especially at the anterior end, and shell coloration is predominantly yellow on the exterior and white on the interior. Age and water quality can produce a darker or more brown exterior shell, and sometimes green rays can be seen on the posterior end. They are ovate in shape with females having a more rounded posterior and males appearing elongated. They have a prominent beak that protrudes above the hinge line. On the interior of the shell they have two pseudocardinal teeth on the left valve, two to three on the right valve, two lateral teeth on the left valve, and one lateral tooth on the right valve (Nedeau 2008). Average longevity of lampsiline species is 15 years (Haag

and Rypel 2011); however, Wick (2006) found that yellow lampmussel longevity may exceed 20 years.

1.5.2 Habitat

Yellow lampmussel are found in medium and large rivers (Strayer and Fetterman 1999, Nedeau et al. 2000, Nedeau 2008); however, in Nova Scotia and Maine they have been found in lakes (MDIFW 2000), COSEWIC 2004, Wick 2006). They are found in sand and fine gravel, from shallow water to areas 10 m deep (Strayer and Jirka 1997, Nedeau 2008). They prefer riffle areas with swift currents; however, yellow lampmussels are also found in slow currents around sand bars in the St. Johns River, New Brunswick (Sabine et al. 2004); Blacketts Lake, Nova Scotia (White 2001); and the Connecticut River, Massachusetts (Nedeau 2008).

1.5.3 Reproduction

Reproductive age of yellow lampmussel is unknown; however, other lampsiline species have been estimated to reach reproductive age between 2 and 4 years depending on growth rate (Haag 2012). During reproduction, males release sperm into the water and females use their inhalant siphon to draw the sperm into their marsupium where eggs become fertilized. Because yellow lampmussel are long term-brooders (bradytictic), similar to other Lampsilini, they are fertilized during the late summer and late fall, then hold their glochidia overwinter into the next spring (Haag 2012). Once a female is fertilized, she is referred to as ‘gravid’. Within gravid females, glochidia develop inside the gill marsupium. After glochidia are sufficiently developed, gravid females display a “lure” that attracts host fish species toward their mantle. If an interested fish approaches the lure or comes into contact with the

underlying marsupia, glochidia are released and attach to the gills of the host fish (Haag and Warren 1999). Host fish species identified for yellow lampmussel include white perch (*Morone americana*), yellow perch (*Perca flavescens*) (Wick and Huryn 2002, Wick 2006, Kneeland and Rhymer 2008), largemouth bass (*Micropterus salmoides*) (Eads et al. 2007), black crappie (*Pomoxis nigromaculatus)* white bass *(Morone chrysops),* and striped bass (*Morone saxatilis) (*Eads et al. 2015). While the glochidia parasitize the fish for 2–4 weeks, they receive vital nutritional resources that aid in the development of internal organs (Fisher and Dimock 2002, Fritts et al. 2013, Douda 2015). Metamorphosis of glochidia to juvenile mussels includes the development of gills, cilia, and stomach, combined with independent pedal foot movement (Roberts and Barnhart 1999). After metamorphosis, the juveniles drop into the substrate where they will continue to develop and grow.

1.5.4 Distribution and Status

The yellow lampmussel is found along the Northeast Atlantic slope of North America, ranging from Georgia (United States) north to Nova Scotia (Canada). As of the latest evaluations, is it considered a species of special concern throughout Nova Scotia and New Brunswick with only one population in the Sydney River watershed, Nova Scotia, one population in the Saint John River watershed, New Brunswick, and one population in Pottle Lake, Nova Scotia (COSEWIC, 2013). In the United States, the yellow lampmussel is threatened, endangered, or imperiled in 9 states (Maine, Massachusetts, Connecticut, New Jersey, Delaware, West Virginia, North Carolina, South Carolina, and Georgia; Table 1.1). In Massachusetts and Connecticut, the yellow lampmussel is listed as endangered and prior to 2006 it was thought to be extirpated from Connecticut. Patches of individuals are only found along an 80 km stretch of the Connecticut River from Windsor, Connecticut to Turners Falls,

Massachusetts and in the Holyoke Power Canals in Holyoke, Massachusetts (Nedeau 2008). In Virginia, yellow lampmussel are not listed as threatened or endangered (Jones 2015), but have been identified as a species of greatest conservation need (VWAP 2015). The species is declining throughout its range and all populations north of New York State are isolated (COSEWIC 2004; Table 2.1). The IUCN Red List classifies the species as vulnerable with decreasing populations (Bogan and Woolnough 2017) and there is evidence that some populations have been hybridizing with congeneric species (Kelly 2004).

1.6 Study Objectives and Thesis Chapters

This thesis aims to fill data gaps regarding culturing techniques for yellow lampmussel. Chapter 2 tests the effect of commercial microbial probiotics on the survival and growth of early stage juvenile yellow lampmussels. Probiotics added to the rearing water may improve growth and survival of early stage juveniles by supplementing nutrition, improving feed efficiency, improving water quality, or boosting the immune system. Specifically, I ask the questions: 1) Does concentration of probiotic affect growth or survival of the mussels? 2) Does type of probiotic used affect growth or survival of the mussels? and 3) How does the use of probiotics affect water quality, and, in turn, growth and survival the mussels? Information disseminated from the experiments using commercial probiotics in juvenile mussel rearing may be applicable to other hatcheries.

Chapter 3 investigates the effect of rearing system design on the survival and growth of juvenile yellow lampmussels by using a surrogate species, the eastern lampmussel (*Lampsilis radiata)*. The objectives of the second chapter are to: 1) compare the growth and survival of juvenile mussels raised in different designed rearing systems, 2) compare the growth and survival of juvenile mussels raised in similar designed rearing systems, at

different culture facilities. Results of this project may be used to inform local management agencies on secondary grow-out and long-term culture of rearing yellow lampmussel

Chapter 4 discusses the conclusions from this thesis and provides recommendations for the yellow lampmussel propagation. Recommendations include genetic management guidelines, brood stock collection, brood stock holding, host-fish inoculation and care, primary juvenile culture, and secondary juvenile culture. Recommendations are based on knowledge gained from previous literature and hands-on experience in the propagation and culturing of yellow lampmussels.

Yellow lampmussel is declining throughout most of its range, and population augmentation may be essential for population persistence. Because propagation has been shown to be an effective tool in freshwater mussel conservation where threats have been mitigated, techniques for successful culture of the species are needed. Collectively, the results of these studies will provide managers with knowledge needed to develop well-informed propagation and rearing protocols for yellow lampmussel.

Table 1.1. State listing status of yellow lampmussel (*Lampsilis cariosa*) in the U.S. and Canada.

Location:	Current River/Watershed	State Listing Status	References
Connecticut	Connecticut River Watershed	Endangered	Nedeau 2008, Connecticut 2014
Delaware	Delaware River Watershed	Endangered possible Extirpated	Delaware 2013, NatureServe 2019
Georgia	Savannah and Ogeechee River Watersheds	Vulnerable	NatureServe 2019
Maine	Kennebec, Saint George, and Penobscot River Watersheds	Threatened	MDIFW 2000, MDIFW 2015
Maryland	Chesapeake Bay Watershed	Unknown*	Bogan and Ashton 2016
Massachusetts	Connecticut River Watershed	Endangered	Nedeau 2008, NHESP 2017
New Brunswick	Saint John River Watershed	Special Concern	COSEWIC 2013
New Hampshire	Extirpated	Extirpated	NatureServe 2019
New Jersey	Delaware River	Threatened	Davenport 2012, NatureServe 2019
New York	Susquehanna, Lawrence, and Hudson River Watersheds	Vulnerable to Apparently Secure	NYNHP 2019
North Carolina	Pee Dee, Cape Fear, Neuse, Tar River Watersheds	Critically Imperiled	NCWRC 2019
Nova Scotia	Sydney River Watershed and Pottle Lake	Special Concern	COSEWIC 2013
Pennsylvania	Susquehanna, Potomac, and Delaware River Watersheds	Vulnerable to Apparently Secure	PFBC 2018
South Carolina	Pee Dee and Broad River Watersheds	Imperiled	Price 2006, Eads et al. 2015
Virginia	Potomac, York, James, Chowan, Roanoke River Watersheds	Not Listed of as threatened or endangered (e.g. no state status), but listed as a species of concern	Jones 2015, VWAP 2015
West Virginia	Potomac River Watershed	Imperiled	WV SWAP 2015, WV Natural Heritage 2016

*"Comment: Thought to be extirpated in Maryland, however current status is unknown because historical and recent collections may have been taxonomically confused with similar species (Bogan and Ashton 2016).

CHAPTER 2
INVESTIGATING THE USE OF PROBIOTICS ON SURVIVAL AND GROWTH OF JUVENILE YELLOW LAMPMUSSEL

2.1 Introduction

Freshwater mussels (family Unionidae) are facing a global extinction crisis. Like other aquatic animals, habitat loss and degradation (Vaughn et al. 1999), biological invasion (Strayer 1999), and land use change (Poole and Downing 2004, Peacock et al. 2005) are some of the major anthropogenic factors affecting mussel populations. Where small population sizes and low dispersal limit freshwater mussel populations, reintroduction and augmentation using propagated mussels has been considered an ideal conservation strategy (NNMCC 1998, FMCC 2016). As such, numerous state and federal agencies have developed laboratory facilities dedicated to freshwater mussel propagation over the last two and a half decades (Patterson et al. 2018).

To optimize survival and growth of freshwater mussels, numerous studies have been conducted to improve propagation and culturing techniques. These studies have resulted in recommendations for various aspects such as rearing system design, diet, disease, and water quality management (summarized in Patterson et al. 2018). One potential strategy to improve culturing success of juvenile mussels is to supplement probiotic bacteria to rearing chambers or water sources. Probiotic bacteria (herein termed "probiotics"), are beneficial bacteria used to improve the animal's health and wellbeing. Improvement in rearing conditions may result from several mechanisms, including: water quality improvements (e.g., via reduced ammonia and nitrate), increased stress tolerance of the animals, nutrition enhancement (including stimulation of digestion and enzymatic processes), and disease prevention through enhanced immune response of host animal,

bacterial production of inhibitory substances (antibacterial, antifungal, antiviral), and competitive exclusion of pathogenic bacteria (synthesized by Cruz et al. 2012, Ibrahem 2013, Pérez-Sánchez et al. 2013, Zorriehzahra et al. 2016).

Probiotics have been used to improve rearing by enhancing health, survival, and growth of several aquatic organisms such as finfish, crustaceans, and marine bivalves (synthesized by Ninewe and Selvin 2009, Prado et al. 2010, Perez-Sanchez et al 2013, Zorriehzahra et al 2016, Chauhuan and Singh 2018); however, information on the use of probiotics in freshwater mussel culture is limited (summarized in Table 2.1). Probiotics have been shown to increase freshwater mussel survival when added to an algal diet (Andy McDonald, USFWS, pers. comm); however, a decrease in mussel survival has also been demonstrated (Gatenby et al. 1996, Vincie 2008). Improvements in growth of freshwater mussels exposed to probiotics has been documented (Gatenby et al 2006, Gill unpublished manuscript 2016; McDonald USFWS, pers. comm, WenYing et al. 2009), however, other studies have shown no effect on mussel growth (Vincie 2008, Zheng et al. 2017). Probiotics have been demonstrated to have a positive effect on metabolic condition, digestive enzyme activity, and immune response (WenYing et al 2009, Bianchi et al 2017) which may translate to improvements in growth, survival, and fitness (Queiroz and Boyd 1998, Wang et al. 2005, Gomez et al. 2007). Differences in response across studies may be due to different probiotic mixes used, or may also be explained by differences in study design, including the sample size and size/age of mussels used. Further studies are needed to clarify differences in results and to determine what probiotic mixes are most effective at improving juvenile mussel growth and survival.

The goal of this study was to investigate the use of probiotics to improve culture of juvenile yellow lampmussel (*Lampsilis cariosa)*. Specifically, I asked whether juvenile mussel survival or growth varies based on probiotic concentration or type. To explicitly test one mechanism of probiotic impacts, I secondarily asked whether water quality differences among probiotic treatments explains juvenile mussel survival or growth responses. To address these questions, I conducted three experiments focused on 1) probiotic concentration (low, medium, and high), 2) probiotic type (six different commercial probiotic mixes), and 3) water quality (achieved by varying water change-out frequency). The results from this study will be used to inform probiotic use in freshwater mussel aquaculture.

2.2 Methods

2.2.1 Study Species

The yellow lampmussel is found along the Northeast Atlantic slope of North America, ranging from Georgia (United States) north to Nova Scotia. They are medium-sized species that can reach a total length of 134 mm (Nedeau 2008). Yellow lampmussel are found in medium and large rivers (Strayer and Fetterman 1999, Nedeau et al. 2000, Nedeau 2008) and sometimes lake or ponds (MDIFW 2000), COSEWIC 2004, Wick 2006). Yellow lampmussel prefers swift current and riffle habitat (Strayer and Jirka 1997, Nedeau 2008) but has been found in slow current around sand bars in New Brunswick (Sabine et al. 2004), Nova Scotia (White 2001); and the Connecticut River of Massachusetts (Nedeau 2008). Average longevity of lampsiline species is 15 years (Haag and Rypel 2011). See Chapter 1 for additional species details.

2.2.2 Study Design

2.2.2.1 Experiment 1: Probiotic Concentration

In the first experiment I exposed juvenile mussels to different concentrations of probiotics to determine if concentration of probiotic affects juvenile growth and survival. Treatments included 3 different concentrations of a probiotic (Alken ClearFlo® 1002, Earth Doctor Inc, Flint, VA) supplement (0.0125 g/L 'Low', 0.025 g/L 'Medium', and 0.05 g/L 'High') mixed with algae, 1 control with no probiotic (Algae Only), and 1 control with no algae and probiotic only (0.025g/L 'Probiotic Only') (Table 2.2). Each treatment had 4 replicates of 400 juvenile mussels (age 1-9 days), except for the algae feed only (n=3).

2.2.2.2 Experiment 2: Probiotic Mix

Juvenile mussels were supplemented with 6 different probiotic mixes to determine if probiotic mix explains growth or survival of the mussels. Treatments include 6 different types of probiotics (Alken ClearFlo® 1000, 1002, 1008, and 1100-50x; NiteOutII© (Ecological Laboratories, Lynnbrook, NY), and Glosso Factory Dry Format Bacteria (Planted Aquarium Concepts LLC, Tempe AZ)) mixed with algae and 1 control with no probiotic (algae only) (Table 2.2). Each treatment had 4 replicates of 500 juvenile mussels (age 14-21 days.), except for one of the treatments (B1000, n=3). For the powder probiotics (B1000, B1002, B1008, and Glosso Factory Dr Format Bacteria) doses were calculated based on similar colony forming units (CFU's).

2.2.2.3 Experiment 3: Water Quality

For the third experiment, I manipulated the frequency of water change-outs to determine if differences in ammonia concentrations explain juvenile growth and survival responses. Treatments included 8 different regiments that varied based on water and feed replacement frequency (Quick = every 2 days, Long = every 6 days), probiotic type, and inclusion of algae feed. The probiotic types were comprised of either genera *Nitrosomonas, Nitrospira,* and *Nitrobacter* (Nitro genera) found in the NiteOut, N1100-50X, and Glosso mixes; or the *Bacillus* genus, and found in the B1000, B1002, and B1008 mixes. The Quick treatments rearing water (feed and/or probiotics) was changed out every other day, and the Long treatments rearing water (feed and probiotics) were changed out every 6th day; and feed and probiotics were supplemented to rearing water every 3rd day. The treatments all had 2–4 replicates of 200–250 juvenile mussels that were primarily 8-14 days old at the start of the experiment (see Table 2.2). Treatment codes in text refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and addition of algae (A). A product description of each probiotic can be found in Appendix A.

2.2.2.3.1 Algae Diet

For all experiments, the algae mixture was a 2:1 ratio (1.5-ml and 0.75-ml) of Marine Microalgae Concentrates Shellfish Diet 1800 ™ and Nanno 3600 ™ (Reed Mariculture Inc., Campbell, CA) to 20 L of sterilized wild water, uniform across treatments. For all experiments the water, algae, and probiotic mixtures were recirculated continuously via a Masterflex® Peristaltic Pump (Cole-Parmer, Vernon Hill, IL). For

experiment 1 the mixtures were continuously recirculated at 8.0 ml/min, for experiments 2 and 3 the mixtures were continuously recirculated at 19.0 ml/min.

2.2.3 Juvenile Mussel Propagation, Collection, and Rearing

2.2.3.1 Gravid Mussel Collection

Juvenile mussels were produced at the U.S. Fish and Wildlife Service's Richard Cronin Aquatic Resource Center (CARC) in Sunderland, Massachusetts. In 2017 and 2018, SCUBA divers collected 30 gravid yellow lampmussels from the Connecticut River in Hadley, Massachusetts. Mussels were transported to CARC where they were placed in 1.5–9 L aquaria with 75-mm of sediment and kept at 5–7°C (see Appendix B for additional collection and holding information).

2.2.3.2 Host Fish Inoculation

Separate host fish inoculations took place in 2017, 2018, and 2019 for the three experiments. Fish inoculations followed standard procedures outlined in Patterson et al. (2018). Glochidia were harvested by piercing mussel gills with a 22-gauge hypodermic needle and flushing glochidia into separate beakers. Glochidia quantity and viability was determined for each female progeny through subsampling and a salt test (Patterson et al. 2018). Mussels with >80% glochidia viability (n = 14 mussels) were used in inoculations. Experiment 1 used glochidia from 6 females; and experiments 2 and 3 used 4 females each.

Largemouth bass (*Micropterus salmoides*) purchased from Hicklings Fish Farm (Edmeston, NY) were used as host fish. Total number of host fish used were 400 (Experiment 1), 450 (Experiment 2), and 210 (Experiment 3). Fish were separated into 4-

8 groups of 13–27 fish and put into 19-liter buckets with 1.5–10 L of water equipped with aeration stones. Glochidia was added to each bucket at 2100–2700 (Experiment 1), 1000 (Experiment 2), 1900–3800 (Experiment 3) viable glochidia per fish. The bass were exposed to glochidia for 25–30 minutes while the water was continually mixed using turkey basters to keep glochidia in suspension. Following inoculation, bass were divided equally into three to four 288-liter. circular tanks kept at an average temperature of 19–20°C for Experiment 1 and 3, and 22-23°C for Experiment 2. Further details of inoculation procedures can be found in Appendix C.

2.2.3.3 Juvenile Collection

Starting the day after inoculations, glochidia and juveniles were siphoned from the bottom of host-fish tanks into 150-µm mesh bags once daily. Additionally, a 100-µm mesh bag was attached to the discharge outlet of each circular tank and checked daily for glochidia and mussels that may have been flushed from the system. Contents of both bags were rinsed through 500-, 250-, and 150-µm mesh to separate debris from glochidia and juveniles. Glochidia and juveniles were transferred to petri dishes and counted using a stereo microscope (magnification 7–30x). Examination of tanks for glochidia and juvenile drop-offs continued until counts dropped to near zero and visual inspection of 5-10 fish from each tank indicated that glochidia were no longer present. Juveniles classified as viable (visible pedal movement; gill and stomach development) were transferred to primary holding chambers.

2.2.3.4 Juvenile Rearing Prior To Experiments

Prior to experiments, juveniles were housed in mini downwelling rearing chambers made of polyvinyl chloride (PVC) units connected to a recirculating system made of a 22-L algae feed bucket, a peristaltic pump for food delivery, and discharge lines (Figure2.1). The rearing chambers were constructed of 150-µm mesh screen that was glued to the bottom of a 10-cm PVC tube that sat inside a 15-cm PVC cap fitted with a feed line and discharge line (Figure 2.2). Mussels (all except no-algae controls) were fed a standard mussel diet of Marine Microalgae Concentrates 1.5-mL Shellfish Diet 1800 and 0.75-mL Nanno 3600 to 20 L of wild-water based on previous feed trials. The source water for all mussels was a mixture of surface and ground water from the fishery raceways located on the property, which was treated with an ultraviolet sterilization light and filtered through 5-µm mesh. A complete water change-out for was done three times a week prior to experiments commencing. An air stone was placed within the feed source and used to keep the water oxygenated. Mussel chambers were sprayed with a garden sprayer each day to free the screen of food and waste by-product buildup. Collected juveniles for Experiment 2 and 3 were housed in similar PVC chambers; however, the individual discharge lines were removed, and chambers were placed together on an overflow pan that discharged directly into the algae feed bucket. Temperature was maintained at approximately 19-21°C for Experiments 1 and 3 and at 24°C for Experiment 2.

For Experiments 1 and 3, juvenile mussels that were dedicated to a probiotic group were started on probiotics beginning immediately after collection from the host fish. For Experiment 2, juvenile mussels were not exposed to probiotics prior to

commencement. Mussels were held until the target number mussels of similar age were collected (Experiment 1: 9 days, 15,000 mussels; Experiment 2: 21 days, 20,000 mussels; Experiment 3: 14 days, 10,000 mussels).

2.2.3.5 Juvenile Rearing During Experiments

Rearing chambers for the experiments were the same as chambers as described for juvenile holding prior to experiments except for Experiments 2 and 3, where the individual discharge lines were removed, and chambers were placed together on an overflow pan that discharged directly into the algae feed bucket. For Experiment 1, a complete water change-out was done once a week and the feed and probiotic mixture was added twice a week, approximately every 3 days. For experiment 2, a complete water change-out was done three times a week, and the feed and probiotic mixture was added with every water change out. For Experiment 3, the water change-out schedule is described in the study design. The source water, temperature, algae feed ratio, and use of aeration stone was the same as juvenile rearing prior to experimentation (e.g. temperatures remained the same at 19-21°C for Experiments 1 and 3 and at 24°C for Experiment 2; and for all Experiments the feed ratio remained at Marine Microalgae Concentrates 1.5-mL Shellfish Diet 1800 and 0.75-mL Nanno 3600 to 20 L of wild-water).

2.2.4 Water Quality

For Experiments 1 and 2, water quality measurements were taken on the day of water change-out, both before replacement (old water) and after replacement (new water). For Experiment 3, water quality was measured daily. For Experiment 1, dissolved

oxygen (DO) and temperature were measured using a YSI™ ProODO Optical DO Meter (YSI Inc./Xylem Inc., Yellow Springs, OH). Salinity, total dissolved solids (TDS), specific conductivity (SPC), and pH were measured using a LaMotte™ Tracer Pocketester (LaMotte Company, Chestertown, MD). Ammonium (NH_4-N) levels were tested using a YSI™ 9300 photometer (YSI Inc./Xylem Inc., Yellow Springs, OH). For Experiments 2 and 3, DO, temperature, SPC, pH, NH_3-N, and NH_4-N were measured using a YSI™ Professional Plus multiparameter meter (YSI Inc./Xylem Inc., Yellow Springs, OH).

2.2.5 Mussel Measurements

2.2.5.1 Survival

Mussels were live-counted once a week for the duration of the experiments (8, 5, 7 weeks respectively). Using a garden sprayer, mussels were removed from the rearing chambers and rinsed through 300 -, 200-, and 150-µm mesh to separate debris from mussels. Mussels were then transferred to petri dishes and counted underneath a stereo microscope (magnification 7–30x). Live mussels were characterized by a combination of foot movement, active gaping or a closed shell, dark stomach contents, and lack of buoyancy. Debris and dead mussel shells were removed from the dish, and live mussels were returned to their respective chambers after being photographed for growth (see below).

2.2.5.2 Growth

All mussels were photographed on the first day of the experiment and once a week thereafter following survival counts to minimize handling. Photographs of the

entire petri dish were taken using Canon® EOS 5DSR camera (Canon USA Inc., Huntington, NY). Using Microsoft Excel® a simple random sample was conducted to select grid cells for juvenile measurement. Juveniles that were laying completely flat with >50% body area within the cell boundaries were then measured posterior to anterior end to the nearest micrometer using Image Pro Insight software (Media Cybernetics, Rockville, MD). The mussels were measured within each grid cells until the target number of mussels per replicate were measured. An *a priori* power-analysis for ANOVA determined that a sample size of 50 mussels per replicate would have a power of 0.90 and detect an effect size of 0.25 with a significance of 0.05 (Experiment 1), a sample size of 50 mussels per replicate would have a power of 0.91 and detect an effect size of 0.23 with a significance of 0.05 (Experiment 2), and a sample size of 50 mussels per replicate would have a power of 0.91 and detect an effect size of 0.22 with a significance of 0.05 (Experiment 3). Power analysis was done to determine effective sample size (R pwr package version 1.2–2).

2.2.6 Data Analysis

All data were analyzed to meet model assumptions (i.e. residuals, homogeneity of variance, normality, deviance, etc.). Non-parametric alternatives were used if assumptions were violated. In all analysis, treatments were treated as categorical factors and days were treated as continuous variables. All statistical tests were calculated with R statistical software (version 3.4.4; R Core Team 2018). All statistical tests were evaluated at the α=0.05 significance.

2.2.6.1 Water Quality

To test for differences in water quality among treatments, water quality parameters (temperature, salinity, TDS, SPC, pH, DO, NH_3-N, and NH_4-N) were analyzed using a one-way analysis of variance (ANOVA) and Tukey's post-hoc analysis; or, if assumptions of normality were not met, non-parametric Kruskal-Wallis and Dunn's rank sum multiple comparison test with Bonferroni correction car package version 3.0–2 and dunn.test package version 1.3.5).

I compared ammonia nitrogen NH_3-N levels for Experiment 3 to the U.S. Environmental Protection Agency's (USEPA) Aquatic Life Ambient Water Criteria for Ammonia (ALAWQCA, USEPA 2013) acute and chronic exposure guidelines. NH_3-N ratios were calculated for the ALAWCA guidelines using the Henderson-Hasselbach equation at specified temperature and pH values (Appendix D).

2.2.6.2 Survival

To test the difference in survival among treatments, a binomial logistic regression model (link=logit) with maximum likelihood estimation was used (R stats package version 3.4.4). Treatment, time, and the interaction of treatment and time were evaluated as significant predictors of survival odds (denotated as odds ratio=OR) for each experiment.

2.2.6.3 Growth

Overall differences in shell length (from the start to end of the experiment) were calculated for each replicate, and treatments were compared using one-way ANOVA. In addition, growth rates were calculated for each replicate each week and for the entire

experiment. Treatments were compared among treatments using one-way ANOVA and Tukeys post-hoc analysis (car package version 3.0–2). Growth rates were calculated as:

$$\frac{\text{shell length at sampling time}^{i} - \text{ shell length at sample time}^{i-1}}{\text{time}^{i} - \text{time}^{i-1}}$$

2.3 Results

2.3.1 Experiment 1

2.3.1.1 Water Quality

There were no significant differences in temperature, dissolved oxygen (DO), or pH among treatments (Table 2.3). Average temperature was 21.8 °C, average DO was 8.31 mg/L and average pH was 7.33. TDS, salinity, and SPC were significantly higher in the High treatment than all other treatments. NH_4-N was significantly higher in Algae Only treatment and was significantly lower in the Medium and High treatment compared to other treatments (Table 2.3).

2.3.1.2 Survival

Mussel survival ranged from 0% to 43.2% over the entire experiment. There were no survivors by day 39 in the Algae Only and by day 46 in the Probiotic Only treatment. Overall survival varied but was not significantly different among the Low (31.3 ± 4.8%), Medium (41.4 ± 5.8%), and High (43.2 ± 17.4%) treatments (Table 2.4, Figure 2.3).

Model comparison for the logistic regression indicates the interaction of treatment*time had a significant effect on survival. Time had a negative impact on survival odds (OR=0.80, P<0.001). The effect of time on survival differed for all treatments when compared to the reference group Algae Only (p<0.001). The ratio of

odds ratios (ROR) indicate that compared to the Algae Only, the Probiotic Only treatment increased odds of survival by a factor of 7% per one-unit change in time. The Low, Medium, and High treatments increased odds of survival by 17%, 18%, and 18% per one-unit change in time, respectively (Table 2.5). Model evaluation is summarized in Appendix E.

2.3.1.3 Growth

Over the entire 53-day experiment, mussels grew from an average of 220 to 237 µm across treatments to an average of 426 to 448 µm (Figure 2.4, Appendix F). Although mussel sizes at day 0 should have been the same across treatments, the average size of the Low treatment was significantly larger all other treatments ($p<0.001$). Thus, I compared the average difference in shell length between day 0 and day 53 for Low (211 ± 37 µm), Medium (200 ± 66 µm), and High (202 ± 28 µm) treatments and found no significant difference ($p=0.733$).

There was a large variability in growth rates among replicates over the duration of the experiment (-2.6 to 8.6 µm/day). Average growth rates peaked within the first 11 days for all treatments except the Low treatment, which peaked between days 33–38 (Figure 2.5). Significant differences in week-to-week growth rates among treatments occurred during the sampling intervals of days 5–11 ($p<0.001$), 12–18 ($p=0.022$), 19–25 ($p=0.004$), 26–32 ($p=0.020$), and 33–38 ($p=0.018$) (Table 2.6). In general, after day 4 the Algae Only and Probiotic Only had lower week-to-week average growth rates than the Low, Medium, and High treatments. At days 5–11, the Medium (7.4 ± 2.1 µm/day) and High (8.0 ± 0.4 µm/day) treatments had significantly higher growth rates than the Low (3.4 ± 1.5 µm/day) treatment. For all other sampling intervals, there were no significant

differences between the Low, Medium, and High treatments (Table 2.6). Overall analysis of the average growth rates among treatments indicate the Algae Only (1.2 ± 3.0 µm/day) and Probiotic Only (1.6 ± 2.4 µm/day) had lower average growth rates than all other treatments (Low, Medium, High), which did not significantly differ from each other (Figure 2.6).

2.3.2 Experiment 2

2.3.2.1 Water Quality

DO, SPC, and NH_4-N were significantly different among treatments (Table 7). The B1000 had the lowest DO (7.86 ± 0.42 mg/L) and was significantly different from all other treatments except the NiteOut and Glosso treatments. The Algae Only had the highest DO (9.16 ± 0.42 mg/L) and was significantly different from the B1000, B1002 NiteOut, and Glosso treatments. The SPC of the Glosso, NiteOut, N1100-50x, B1008 and Algae Only were similar with an average of 156 µS/cm, and significantly lower than both the B1002 (174 ± 24.2 µS/cm) and B1008 (250 ± 35.5 µS/cm) treatments. NH_4-N was significantly higher in the B1000 (0.158 ± 0.28 mg/L) and B1008 (0.121 ± 0.021 mg/L) treatments compared to other treatments. Kruskal-Wallis test indicated significant differences in un-ionized ammonia values (NH_3-N mg/L) between treatments; however, Dunn's test failed to verify statistical differences. There were no significant differences in temperature or pH between treatments (Table 2.7).

2.3.2.2 Survival

At day 51, survival of the juvenile mussels ranged from 0% to 11.1% (Table 2.8, Figure 2.7). By day 30, there were no survivors in the Algae Only, B1002, N1000-50X,

or Glosso treatments; and at day 51 there were only survivors in the B1000 (11.1 ± 5.2%) and NiteOut (0.9 ± 1.1%) treatment. Survival of B1000 and NiteOut differed significantly ($p<0.001$) after 51 days.

Model comparison indicates the interaction of treatment*time had a significant effect on survival. Time was seen to have a negative impact on survival odds (OR=0.77, $P<0.001$) although the effect of time on survival differed for all treatments. Compared to the Algae Only, the NiteOut and B1000 treatments increased probabilities of survival by a factor of 10% and 19%, per one-unit change in time. The N1100-50X, Glosso, B1002, and B1008 decreased the odds of survival by 2%, 5%, 9%, and 38% per one-unit change in time, respectively (Table 2.9). Model evaluation is summarized in Appendix E.

2.3.2.3 Growth

At day 0, mussel size ranged from 406 to 423 µm among treatments, with no significant differences among treatments (Figure 2.8, Appendix F). Average difference in shell length between day 0 and day 51 for B1000 (306 ± 12 µm) and NiteOut (285 ± 97) µm were not significantly different ($p=0.681$).

Growth rates among replicates ranged from -2.3 to 16.2 µm/day over the duration of the experiment. All treatment average growth rates peaked between day 0 and 7, except the N1100-50X which peaked during days 17–23; and the B1000 which peaked between days 31–-37 (Figure 2.9). Week to week growth rates were similar among treatments for all sampling intervals except days 24–30 ($p=0.0374$) where B1008 (-0.43µm/day) had a significantly lower growth rate than B1000 (6.4 ± 1.0 µm/day); however, the B1008 growth rate was calculated from 1 replicate with only 4 mussels (Table 2.10). The B1000 and NiteOut treatments were not significantly different from

each other (Table 2.10). Over the entire experiment, the lowest average growth rate was B1002 (3.0 ± 0.82 µm/day) and the highest average growth rate was B1000 (5.7 ± 2.2 µm/day), and there were no significant difference among treatments (p=0.262, Figure 2.10).

2.3.3 Experiment 3

2.3.3.1 Water Quality

DO, pH, SPC, NH_4-N, and NH_3-N were significantly different among treatments (Table 2.11). LAC had the lowest DO concentration (7.24 ± 0.88 mg/L) and LNA had the highest DO concentration (7.94 ± 0.84 mg/L). Average DO decreased between day 7 and 14, but stabilized afterwards (Appendix G). pH ranged from 7.48 (QN) to 7.71 (LBA). QN had the lowest average SPC at 142 ± 0.87 µS/cm and LBA had the highest average SPC at 200 ± 14.1 µS/cm. Individual plot points of SPC each day revealed an increase in daily measurements during each 6 day period for the Long treatments, where no pattern is evident in the Quick treatments (Appendix G). Average NH_3-N ranged from 0.003 ± 0.004 mg/L (QN) to 0.011 ± 0.007 NH_3-N mg/L (LBNA). LNA (0.011 ± 0.007 NH_3-N mg/L) and LBNA (0.010 ± 0.008 NH_3-N mg/L) had similar concentrations of NH_3-N, and both were significantly higher from all other treatments (Figure 2.11). Average NH_3-N appeared to increase over time, except for LBNA, which decreased at day 20 (Appendix G). Average NH_4-N ranged from 0.285 ± 0.081 mg/L (QAC) to 0.581 ± 0.188 NH_4-N mg/L (LNA). Average NH_3-N and NH_4-N increased over time except for LBNA, which decreased at day 20 (Appendix G). All average temperatures exhibited a similar pattern

over time, with an average increase in temperature over the first two weeks by 0.5–1.5 °C, then an oscillating (higher/lower) average each week afterwards (Appendix G).

2.3.3.2 Survival

Survival over the 35-day experiment varied among treatments: QNA (25.2 ± 5.9%), QAC (22.1 ± 10.0%), LAC (21.7 ± 6.1%), LNA (18.9 ± 6.8%), QBA (3.9 ± 0.9%), and LBA (2.5 ± 1.5%), and differed significantly among treatments (Table 2.12 Figure 2.12). At the end of 35 days, QNA and LBA had lower survival than QNA, QAC, LAC, and LNA. There were no survivors in the QN or LBNA treatment.

Compared to the LAC, the QAC and QNA treatments increased probabilities of survival by a factor of 1% per one-unit change in time; however, they were not significantly different from LAC. For all other treatments, survival decreased when compared to the Algae Only. Probabilities of survival decreased by factor of 3% LNA, 9% QBA, 12% LBA and 29% QN and LBNA per one-unit change in time (Table 2.13). Model evaluation is summarized in Appendix E.

2.3.3.3 Growth

At day 0, average juvenile sizes ranged from 231 to 323 µm with several significant differences between average sizes ($p<0.001$). Because there were differences in day 0 treatment size, I compared differences in shell length between day 0 and day 35 among treatments. LAC (251 ± 6 µm) had the highest difference in shell length and was significantly different than all other treatments except QAC (200 ± 23 µm). LNA (92 ± 22 µm) had the lowest difference in shell length than all other treatments, except QNA (125 ± 8 µm) (Figure 2.13. Appendix F).

There was a large variability in growth rates among replicates over the duration of the experiment (-3.7 to 15.3 µm/day). Peak growth rates differed by treatment, with no observable patterns across treatments (Figure 2.14). There were significant differences in week-to-week growth rates during sampling at days 8–14 and 22–28 (Table 2.14). At sampling interval days 8–14, LAC (10.5 ± 1.5 µm/day) had the highest growth rate and QN (1.0 ± 0.14 µm/day) had the lowest growth rate. At sampling interval days 22–28, the highest growth rate was LBA (12.2 ± 5.1 µm/day) and the lowest growth rate was QNA (-1.3 ± 1.6 µm/day) (Table 12). At sampling interval days 0–7 the highest growth rate was QAC (4.2 ±0.72 µm/day) and the lowest growth rate was QN (-1.5 ± 0.40 µm/day). ANOVA indicated a significant difference between treatments; however, Tukey's post-hoc analysis failed to verify statistical differences. Overall analysis of average growth rates indicates significant difference among treatments (p=0.007) (Figure 2.15). Average growth rates over the entire experiment in increasing order were: QN (-0.17 ± 0.18 µm/day), LBNA (1.2 ± 1.5 µm/day), LNA (2.2 ± 2.0 µm/day), QNA (3.0 ± 1.6 µm/day), LBA (4.3 ± 3.2 µm/day), QBA (4.3 ± 3.5 µm/day), QAC (4.8 ± 1.9 µm/day) and LAC (6.0 ± 1.6 µm/day).

2.4 Discussion

2.4.1 Overall Findings

The effect of probiotics on growth and survival of juvenile mussels were variable across experiments, which was consistent the previous literature on probiotics that showed different effects among studies (see Table 2.1). In the first experiment, the addition of probiotic to an algae diet improved growth and survival responses; however,

probiotic concentration did not affect survival or growth, so any concentration within the doses applied (0.0125–0.05 g/L) should result in benefits. In Experiment 2, two of the probiotic types tested (B1000 and NiteOut) improved survival; however, the probiotic tested in Experiment 1 (B1002) did not increase growth or survival in this study. Overall survival in Experiment 2 was very low and probiotic type did not affect average growth rates. In Experiment 3, I found that treatments with lower NH_3-N concentrations had the highest growth rates; however, the relationship between NH_3-N concentration and survival was less clear. Overall, NH_3-N concentrations were low and concentrations were not lowest in the probiotic treatments (as opposed to those without probiotics); thus, improved water quality did not appear to be the mechanism of probiotic benefit.

2.4.2 Does probiotic concentration affect survival or growth of juvenile mussels? (Experiment 1)

Probiotics improved both the growth and survival of the juvenile mussels when added to the regular algae diet; however, concentration of probiotics did not result in differences of survival or growth. The probiotic used in experiment 1, Alken Clear-Flo 1002 (B1002), has previously been tested in one formal study. The survival and growth results of Experiment 1 are contradictory to Vincie (2008) who found lower growth and lower survival rates of mussels using the same probiotic, compared to other treatments. A possible reason for differences in survival and growth between studies may be the nutritional requirements of the species of mussel used. While bacteria has been shown to be an important food resource for freshwater mussels (Nichols and Garling 2000, Raikow and Hamilton 2001, Vaughn et al. 2008), differences in food preference among species (Bisbee 1984, Nichols and Garling 2000), differences in gill morphology among species

(Silverman et al. 1997), and species-specific number of latero-frontal cirri (Owen and McCrae 1976) suggest different species may have different food requirements. Juvenile mussels in this study were of the genus *Lampsilis* while juveniles used by Vincie (2008) were of the genus *Epioblasma*, which may explain differences in survival and growth among studies.

Higher probiotic concentrations did not significantly improve growth or survival of juvenile mussels over lower concentrations. Similar survival and growth rates among the Low, Medium, and High treatments may be explained by the generational time of the bacteria combined with clearance rate of bacteria by mussels. *Bacillus* species have a doubling time (generational growth) rate of 30-120 min (Collins and Richmond 1962, Burdett et al. 1986) and clearance rate of bacteria is directly related to animal size, gill surface area and number, and size of cirra and cilia per cirral plate (Silverman et al. 1997). *Lampsilis ovata* has been shown to remove bacteria from rearing water at a rate of 5.9 ml/mussel g^{-1} dry tissue/min^{-1}; or 3.21 µL/mm^{-2} gill area/min^{-1} based on gill surface area (Silverman et al. 1997); however, these estimates are for mussels that are primarily filter feeding; whereas juvenile mussels are predominantly pedal feeding. Regardless, if bacteria generation time outpaces mussel clearance rates, then bacterial availability as a food resource would not be considered a limiting factor in survival or growth.

Growth and survival were lowest in the Algae Only and Probiotic Only treatments compared to the algae and probiotic treatments, suggesting that both algae and bacteria are needed to fulfill the nutritional requirements of freshwater mussels. Algae is a main food source for freshwater mussels, providing protein and carbohydrates for primary energy production (White et al, 1989, Nichols and Garling 2000); and commercial algae

diets are regularly used to feed mussels in hatchery settings (Henley et al. 2001, Vincie 2008, Hua et al. 2013, Mair et al. 2013, Patterson et al. 2018). However, rearing systems using natural water with organic detritus or biofloc materials (e.g. bacteria species, filamentous organisms, fungi, protozoans, and metazoans) often provide better culture conditions, thus improving survival and growth for juvenile mussels (Beaty 2004, Beck and Neves 2004, Kovitvadhi et al. 2006, Vincie 2008). In some riverine habitats mussels have been shown to rely heavily on bacteria as a food resource (Raikow and Hamilton 2001, Vaughn et al. 2008). The Probiotic Only treatment had high survival through the first half of the study, which may be due in part to bacterial content and lipid energy reserves obtained during host-fish encystement (Lasee 1991). After the host-derived energy reserve was depleted, bacterial content alone may not have been nutritionally adequate for mussel survival.

Survival and growth of the Probiotic Only and Algae Only treatments could have been due to differences in water quality, especially where probiotics were not used; however, all water quality analysis results were within tolerable range for freshwater mussels, therefore water quality does not appear to be a source of mortality.

2.4.3 Does type of probiotic affect survival or growth of juvenile mussels? (Experiment 2)

I found that the mix of probiotic affected survival of the juvenile mussels when added to the regular algae diet, but did not have an effect on average growth rates; and that one mix of each genus-type of probiotic increased the odds of survival compared to the Algae Only treatment. The B1000 mix of the genus *Bacillus* and the NiteOut mix of the Nitro genera (*Nitrosomonas, Nitrospira,* and *Nitrobacter*) improved odds of survival,

but had no effect on growth rates indicating that there is no difference between using different genus types. All genera of bacteria that were used are considered nitrifying agents, and though different in genera classification, bacterial make-up of the probiotics share similarities in shape (rod or pear shaped) and size (range: 0.5-4.0L X 0.3-2.0W) (Appendix A). Freshwater mussels are able to selectively feed on algae based on characteristics such as shape and size (Paterson 1984, Beck and Neves 2003); therefore, similarities between probiotic make-up may explain the lack of difference between genus.

By day 30, there were no surviving mussels in 4 of the 7 treatments. Time was found to be a significant factor in odds of survival, as observed by the difference in survival times for each treatment and demonstrated by logistic regression analysis. The high mortality observed is typical of juvenile mussels in the first 60 days (Gatenby et al. 1996, Rogers 1999, Beck and Neves 2003, Beaty and Neves 2004, Vincie 2008, Hua et al. 2013). Early mortality of juvenile mussels may be attributed to food quantity and nutritional content (Yeager et al. 1994, Gatenby et al 1996), ammonia concentration (Augspurger et al. 2003, Newton and Bartsch 2007), thermal limits (Pandolfo et al. 2010), or predation (Hanlon 2000, Zimmerman et al. 2003). The B1000 and NiteOut treatments were the only treatments with remaining juveniles at day 51, but were still declining each week, indicating that other factors were continuing to exert pressure on the juveniles causing mortality. Water quality was within normal ranges for freshwater mussel rearing, and predation by flatworms was not observed during this study; therefore, does not seem to be a likely source of mortality.

It appears that the N1100-50X, Glosso, B1002, and B1008 were not beneficial to juvenile mussel survival, and, in fact, decreased the odds of survival compared to the

Algae Only. These results are contradictory to Experiment 1, in which the B1002 improved the odds of survival significantly over the Algae Only treatment, yet are similar to those obtained by Vincie (2008) where the same probiotic mix was attributed to low survival. One explanation for the difference between studies using the same probiotic may be the timing (mussel age) of probiotic administration. In Experiment 1, juveniles were exposed to probiotics on day 1 of juvenile drop-off; whereas in Experiment 2 juveniles were not exposed to probiotics until the experiment started (7-14 days after drop-off). Similarly, juveniles used by Vincie (2008) were approximately 21 days old before probiotic exposure. Exposure to probiotics on day 1 may have a significant influence on survival; especially if the probiotics enhance immune response to bacterial pathogens as seen in numerous other aquatic animals (Sharifuzzaman Austin 2017, Hoseinifar et al. 2018). Knowledge of freshwater mussel bacterial pathogens is in its infancy (reviewed in Grizzel and Brunner 2009, Carella et al 2016) and identification of bacteria that are pathogenic is particularly hard because 1) the bacterial community present in a mussel changes after mortality, 2) bacteria are commonly present in the animal because they are an important food resource, and 3) virulence of bacterial pathogens can change between strains and between mussel species (Lane and Birbeck 2000, Allem et al. 2006, Grizzel and Brunner 2009); however, the high mortality of about 50% of 6/8 treatments within the first week is characteristic of pathogens in marine mussels (Kesarcodi-Watson 2009).

Mortality within the B1008 and Glosso treatments may have been due to a combination of a slimy biofilm and increased turbidity reducing feeding efficiency. Biofilm produced by gram negative nitrifying bacteria prefer to colonize plastic and

PVC-type materials (Vess et al. 1993); and act as an underwater adhesive (Zardes et al. 2008, Hadfield 2011). Newly metamorphosed juvenile mussels move around as they pedal-feed; therefore, the biofilm may interfere with feeding efficiency reducing movement. The effect of biofilms is better understood in marine mussels where biofilms cue larvae life-long settlement for many species (Hadfield and Paul 2001); however, the effect of biofilms on juvenile freshwater mussels has not previously been studied. It is important to note that the NiteOut and N1100-50X treatments were also gram-negative nitrifying bacteria; however, there was no noticeable biofilm in the culture system. Additionally, total suspended solids were not measured in this study, but I observed higher turbidity in the B1008 and Glosso treatments. This may have been due to the physical make-up of the B1008 and Glosso probiotics, which had a base of powders which were semi-soluble and left un-dissolved debris in the water column; whereas, other powdered probiotics (B1000 and B1002) appeared more soluble leaving little residue in the water column. Suspended solids have been shown to reduce clearance rates in juvenile mussel feeding efficiency (Tuttle-Raycraft et al. 2018).

2.4.4 Do probiotics reduce ammonia (NH_3-N), and, in turn, improve survival or growth or juvenile mussels? (Experiment 3)

Ammonia (NH_3-N), is toxic to aquatic animals at small concentrations (Augspurger et al. 2003), affecting valve responses (Epifanio and Srna 1975), byssal thread production (Reddy and Menon 1979), and metabolic processes (Chetty and Indira 1995), which ultimately affect growth and survival (Goudreau et al. 1993). Because probiotics contain denitrifying bacteria that may reduce NH_3-N concentrations (Cruz et al. 2012), I manipulated water change-outs to evaluate how differences in NH_3-N

concentrations among treatments may affect juvenile mussel growth or survival. I expected to find that treatments with probiotics (QNA, LNA, QBA, LBA, QN, LBNA) had lower NH_3-N concentrations than treatments without probiotics (QAC and LAC); and treatments on the "Quick" change-out schedule (QAC, QNA, QBA) were similar in NH_3-N concentration, yet lower than those with no probiotics (LAC, QAC). I also expected to find that treatments on the "Long" change-out schedule (LNA, LBA, LBNA) with probiotics were similar to "Quick" change-out with probiotics (QBA, QNA) and lower than "Long" change-out (LAC) without probiotics. Furthermore, because QN had no algae and only probiotics, I expected to find that NH_3-N concentration to be the lowest in this treatment. In this experiment, probiotics did not significantly reduce NH_3-N concentration and that concentration of NH_3-N was highest in LNA and LBNA while concentrations of NH_3-N were the lowest in the QN, QAC and LAC treatments.

Both control treatments with no probiotics (QAC, LAC) and both NiteOut mixes (LNA, QNA) had the highest observed survival and similar survival compared to the LAC diet. The high survival of the QAC and LAC treatments contradicts results obtained in the first experiment where probiotic and algae diets provided significantly higher survival and growth over an algae only diet. Higher survival from an Algae Only diet (vs one with a algae and probiotics) is supported by Gatenby et al (1996). QAC and LAC also had higher growth rates compared to the other treatments, which is similar to results reported by Vincie (2008) in which a highly concentrated algae-only diet had the highest growth rate compared to probiotic treatments. Food quantity per mussel was higher in Experiment 3 than in Experiment 1 or 2. Mussels habituating lentic or highly produce lotic environments may rely predominantly on algae and phytoplankton as a primary food

source (Vaughn et al. 2008), which suggests that if there is a sufficient quality and quantity of algae available, bacteria may be ingested as a secondary food resource. Therefore, algae food quantity in Experiment 3 may help explain the lack of survival and growth responses in Experiment 3 compared to Experiment 1.

NH_3-N concentration may help explain growth responses, although the relationship between NH_3-N and growth was not directly tested in this study. QAC and LAC had higher growth rates and the lowest average NH_3-N concentrations, and LNA and LBNA had the lowest average growth rates among treatments and highest average NH_3-N concentrations among treatments. The effective toxic concentration (EC50) of NH_3-N for freshwater mussels is 0.03 mg/L, which is the concentration that will reduce growth in 50% of exposed freshwater mussels (Newton and Bartsch 2006). All sampled NH_3-N values remained below 0.03 mg/L and were well below USEPA guidelines for both acute and chronic exposure (USEPA 2013); however, design of the culturing containers may have impacted NH_3-N concentrations as accumulation of NH_3-N at the screen-water interface may have been higher than the sampled values (Newton et al. 2017). While other water quality parameters (temperature, DO, SPC, and pH) had some differences between treatments, all values are considered under the normal parameters for culture of freshwater mussels.

2.4.5 Differences in Responses to Probiotics Among Experiments

This study indicates mixed results in the use of probiotics, much like previous literature (summarized in Table 2.1); however, there are several reasons why the results of these three experiments differed. Given we would expect a similar response between treatments and experiments using the same probiotic, the results suggest differences

between experiments may be due to factors outside of the experimental design. While the same species of mussel was used for all 3 experiments, brood stock collection, brood stock/glochidia holding, age of juveniles used, and pre-exposure to probiotics differed among treatments. Glochidia maturity at host infestation has been found to be a significant factor in post-metamorphosis survival. Jones et al (2005) observed that when glochidia were harvested in the fall of the reproductive year the glochidia were not fully mature and metamorphosed juveniles were less active pedal-feeders than juveniles produced the following spring, decreasing growth and survival compared to juvenile produced (Jones et al. 2005). Brood stock was collected at three different times of year: June, October, and December. Jones et al (2005) observed that when glochidia were harvested in the fall of reproductive year the glochidia were not fully mature, and metamorphosed juveniles were less active pedal-feeders decreasing growth and survival. Among all juveniles produced, glochidia harvested from brood stock collected in October and December had lower overall survival rates than those produced from glochidia harvested in June. Brood stock was also held for different times before being used for host-fish inoculation. The shortest holding time was 5 weeks (Experiment 1) whereas the longest holding time was 24 weeks (Experiment 2). A reduction in feeding during this time may have impacted the glochidia development and decreased fitness of the individuals (Silverman et al. 1987, Tankersly 1996, Schwartz and Dimock 2001). During development, glochidia acquire nutrients, glycogen, lipids, carbon, and calcium from the maternal brood (Silverman et al. 1987, Schwartz and Dimock 2001). During the winter, burrowing and brooding mussels have reduced clearance rates and feeding efficiency compared to normal feeding behaviors during the spring and summer (Tankersly 1996),

suggesting that if a gravid mussel were deprived of food resources during glochidia brooding (pre-winter burrowing), then glochidia fitness may decrease compared to glochidia from actively feeding adults. Finally, encystment duration and host-fish condition have been shown to affect post-metamorphosis survival. Longer, low temperature encystment periods led to higher survival and growth of post-metamorphosed juveniles (Marhawa et al. 2016) and condition of host fish affects survival and growth (Österling and Larson 2013, Douda 2015). In Experiment 3, the glochidia host-fish encystment phase was shorter because temperature of the fish culture were higher compared to Experiment 1 and 2 inoculations. Therefore, earlier brood stock collection, reduced feeding during brood stock holding, host-fish encystment could explain reduced survival and growth responses seen among experiments using similar treatments.

2.5 Conclusion

Juvenile mussels have an extremely high mortality rate at young ages (< 4 months) and small sizes (<1.0 mm; Patterson et al. 2018). Although such high mortality rates of early age juveniles may be "par for the course", improvement in survival and growth, even on a small scale, can have significant impacts on mussel production. Probiotics have improved survival and growth in several other aquaculture practices, and therefore have the potential to positively impact the culture of freshwater mussels. This study provided valuable information concerning the use of commercial probiotics in freshwater mussel culture. I have identified that "more" does not necessarily mean "better" as different levels of probiotic concentration provided similar survival and

growth responses in mussel culture. Additionally, the necessity of both bacteria and algae in a diet was evident in the first experiment. Growth responses did not differ by probiotic type or mix; however, two probiotic mixes that were tested improved survival compared to an algae only diet. Finally, probiotic use did not significantly reduce NH_3-N among treatments; however, higher NH_3-N may have reduce growth rates, though this relationship was not explicitly tested. The findings of this study mirror findings of previous literature where substantially different results in survival and growth have been attained by using probiotic.

These results highlight the need for additional research into probiotic use. Future work stemming from this study should include determining if juvenile mussels have higher survival and growth rates if exposed to probiotics early after dropping off the host-fish. Understanding the relationship between bacteria (or probiotics) in the laboratory is important to optimize survival and growth of cultured freshwater mussels and will aid in population restoration of endangered species.

Table 2.1. Review of probiotic studies in freshwater mussel culture. ACF=Alken Clear-Flo and n.d. = no data available

Mussel Species	**Initial shell length (mm)**	**Initial weight (grams)**	**Probiotic**	**Duration (days)**	**Response**	**Reference**
Diplodon chilensis	66.67	24.72	*Euglena gracilis*	90	Improvement in metabolic condition, digestive enzyme activity, and immune response; no improvement of oxidative balance	Bianchi et al. 2017
Epioblasma capsaeformis	0.525	n.d.	ACF-1002	51	No improvement in growth or survival	Vincie 2008
Hyriopsis cumingii	85.2	56	*Bacillus licheniformis*	30	Improvement of digestive enzyme activity, antioxidant metrics, immune response, and weight gain	WenYing et al. 2009
Hyriopsis cumingii	n.d.	206.9	Novozymes Pond Protect, Bio-Form BZT- Water Reform, Effect Microbes, *Bacillus natto*	80	No improvement in weight gain, or water quality for any tested probiotic	Zheng et al. 2017
Lampsilis cardium	3.0	n.d.	ACF-1002, 1006, and 1008	30	1002 increased growth and survival over 1006 and 1008 mixes	McDonald, A (personal comm.).
Lampsilis radiata	0.725	n.d.	*Geobacter sulfurreducens*	35	Increased growth, effect on survival undetermined	Gill et al. (manuscript in preparation)
Pyganadon grandis	0.367	n.d.	Aqua Bacta-Aid	45	No improvement in growth or survival	Gatenby et al. 1996
Villosa iris	0.25	n.d.	Aqua Bacta-Aid	45	No improvement in growth or survival	Gatenby et al. 1996

Table 2.2. Summarized treatment details of each experiment. Mussel cohort age is age of yellow lampmussel (*Lampsilis cariosa)* mussels at start of experiment. Probiotic name is the commercial probiotic source. ACF=Alken Clear-Flo probiotic. Dosage per liter is in grams (power) or milliliters (liquid); colony forming units (CFU) are not available for liquid probiotics (denotated as N/A). For all experiments, the algae mixture was a 2:1 ratio (1.5-ml and 0.75-ml) of Marine Microalgae Concentrates Shellfish Diet 1800 ™ and Nanno 3600 ™ (Reed Mariculture Inc., Campbell, CA) to 20 L of sterilized wild water, uniform across treatments

Treatment Name	**Replicates**	**Individuals per replicate**	**Mussel Cohort Age (days)**	**Probiotic Name**	**Form**	**Dosage**	**Total CFU/20L**
Experiment 1							
Algae Only (Control)	3	400	1-9	-	-	-	-
Low	4	400	1-9	ACF 1002	powder	0.0125 g/L	$5.0x10^{9}$
Med	4	400	1-9	ACF 1002	powder	0.025 g/L	$1.0x10^{10}$
High	4	400	1-9	ACF 1002	powder	0.05 g/L	$2.0x10^{10}$
Probiotic Only (Control)	4	400	1-9	ACF 1002	powder	0.025 g/L	$1.0x10^{10}$
Experiment 2							
Algae Only (Control)	4	500	14-21	-	-	-	-
B1000	3	500	14-21	ACF 1000	powder	0.125 g/L	$5.0x10^{9}$
B1002	4	500	14-21	ACF 1002	powder	0.025 g/L	$5.0x10^{9}$
B1008	4	500	14-21	ACF 1008	powder	0.0625 g/L	$5.0x10^{9}$
N1100-50x	4	500	14-21	ACF 1100-50x	liquid	0.38 ml/L	N/A
NiteOut	4	500	14-21	NiteOutII	liquid	0.125 ml/L	N/A
Glosso	4	500	14-21	Glosso Factory	powder	0.0295 g/L	$5.0x10^{9}$
Experiment 3							
Quick Algae Control (QAC)	3	250	8-14	-	-	-	-
Quick *Bacillus* Algae (QBA)	3	250	8-14	ACF 1002	powder	0.025g/L	$1.0x10^{10}$
Quick Nitro Algae (QNA)	3	250	8-14	NiteOutII	liquid	0.125 ml/L	N/A
Quick Nitro Only Control (QN)	2	200	1-6	NiteOutII	liquid	0.125 ml/L	N/A
Long Algae Control (LAC)	3	250	8-14	-	-	-	-
Long *Bacillus* Algae (LBA)	3	250	8-14	ACF 1002	powder	0.025g/L	$1.0x10^{10}$
Long Nitro Algae (LNA)	4	250	8-14	NiteOutII	liquid	0.125 ml/L	N/A
Long *Bacillus* Nitro Algae Mix (LBNA)	4	250	8-14	ACF 1002 + NiteOutII	powder+liquid	0.025 g/L + 0.125 ml/L	N/A

Table 2.3. Water quality analysis for Experiment 1 (mean ± SD). TDS=Total Dissolved Solids. SPC= Specific Conductivity. Means followed by a common letter are not significantly different ($p<0.05$). Analysis method indicates the use of ANOVA (A) or Kruskal Wallis (KW) and Tukey's Post Hoc (T) when appropriate. Bold indicates significant group differences ($p<0.05$) and asterisk(*) indicates different sample sizes for one or more analysis. For Ammonium, Algae Only (Control) n=6; Low, Med, High, and Probiotic Only (Control) n=13.

Treatment Name	**n**	**Temp (°C)**	**DO (mg/L)**	**pH**	**TDS (ppm)**	**Salinity (ppm)**	**SPC (µS/cm)**	**Ammonia Nitrogen (mg/L N)**
Algae Only (Control)	8*	21.4 ± 1.47	8.37 ± 0.30	7.28 ± 0.9	91.2 ± 21.7^{a}	57.5 ± 11.6^{a}	136± 20.8^{a}	0.085 ± 0.046^{a}
Probiotic Only (Control)	12*	22.1 ± 1.82	8.37 ± 0.41	7.33 ± 0.12	106 ± 17.5^{a}	66.7 ± 13.0^{a}	159 ± 36.8^{a}	0.043 ± 0.440^{ab}
Low	15*	21.4 ± 2.63	8.40 ± 0.43	7.35 ± 0.14	104 ± 13.2^{a}	64.7 ± 9.15^{a}	158 ± 30.7^{a}	0.042 ± 0.038^{ab}
Med	15*	22.5 ± 2.05	8.17 ±0.0.43	7.35 ± 0.12	116 ± 17.0^{a}	76.7 ± 14.0^{a}	176 ± 40.0^{a}	0.019 ± 0.016^{b}
High	15*	22.0 ±1.90	8.26 ± 0.34	7.34 ± 0.11	144 ± 25.3^{b}	94.7 ± 19.2^{b}	217± 55.1^{b}	0.018 ± 0.015^{b}
Analysis Method		K	K	T	T/A	T/A	T/A	T/A
Group Pr(>F)		0.367	0.6002	0.679	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Table 2.4. Observed survival (mean ± SD %) for Experiment 1 yellow lampmussel (*Lampsilis cariosa).* Raw data averaged from four replicates (n) per treatment (except Algae Only (Control) which had three replicates). Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$).

	Sampling Days								
Treatment	**0**	**4**	**11**	**18**	**25**	**32**	**39**	**46**	**53**
Algae Only (Control)	100 ± 0	67.4 ± 5.5^{a}	36.9 ± 7.4^{a}	16.9 ± 5.8^{a}	6.3 ± 2.7^{a}	0.5 ± 0.6^{a}	0 ± 0^{a}	0 ± 0^{b}	0 ± 0^{b}
Probiotic Only (Control)	100 ± 0	90.2 ± 3.6^{b}	82.5 ± 3.9^{b}	76.1 ± 8.9^{b}	70.4 ± 6.4^{b}	36.1 ±5.8^{b}	4.13 ± 2.1^{a}	0 ± 0^{b}	0 ± 0^{b}
Low	100 ± 0	94.4 ± 3.8^{b}	71.3 ± 5.7^{b}	62.6 ± 5.0^{b}	54.8 ± 4.9^{b}	46.1 ± 2.9^{b}	39.7 ± 2.4^{b}	32.3 ± 5.5^{a}	31.3 ± 4.8^{a}
Med	100 ± 0	90.3 ± 3.4^{b}	77.2 ± 10.1^{b}	70.1 ± 11.4^{b}	63.1 ± 12.8^{b}	56.1 ± 10.6^{b}	50.5 ± 5.7^{b}	45.0 ± 5.0^{a}	41.4 ± 5.8^{a}
High	100 ± 0	94.2 ± 2.3^{b}	79.8 ± 3.3^{b}	73.7 ± 5.3^{b}	60.1 ± 9.5^{b}	52.9 ± 13.4^{b}	46.4 ± 19.3^{b}	45.9 ± 20.1^{a}	43.2 ± 17.4^{a}
Group Pr(>F)		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.268	0.290

Table 2.5. Logistic regression analysis results of survival data for Experiment 1 juvenile yellow lampmussel (*Lampsilis cariosa*). Odds ratio indicate change in survival odds (in percent) compared to the reference (intercept) group.

Treatment	Log Odds	Odds Ratio	SE (Odds Ratio)	2.5%-97.5% Confident Interval (Odds Ratio)	Odds Ratio (Percent Change)	z-value	Pr>χ^2
Algae Only (Control)- Intercept	2.11	8.24	0.482	7.35-9.25		36	<0.001
Probiotic Only (Control)	1.78	5.92	0.518	5.0-7.0		20.4	<0.001
Low	-0.03	0.97	0.069	0.85-1.12		-0.4	0.692
Medium	0.02	1.02	0.075	0.89-1.17		0.28	0.785
High	0.15	1.16	0.083	1.01-1.33		2.1	0.039
Time- Intercept	-0.22	0.80	0.004	0.80-0.81	-20.00	-45.6	<0.001
Probiotic Only (Control)*Time	0.07	1.07	0.006	1.06-1.08	7.00	12.6	<0.001
Low*Time	0.15	1.17	0.006	1.16-1.18	17.00	31.5	<0.001
Medium*Time	0.16	1.18	0.006	1.17-1.19	18.00	33.8	<0.001
High*Time	0.16	1.18	0.006	1.16-1.19	18.00	33	<0.001
Null deviance: 35821 on 170 degrees of freedom Residual deviance: 4552 on 161 degrees of freedom AIC:5374							

Table 2.6. Mussel growth rate (mean ± SD µm/day) for Experiment 1 juvenile yellow lampmussel (*Lampsilis cariosa)*. Raw data averaged from four replicates (n) per treatment (except Algae Only Control which had three replicates). Bold indicates significant group difference using one-way ANOVA ($p<0.05$). Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$).

		Sampling Interval (Days)								
Treatment	**n**	**0-4**	**5-11**	**12-18**	**19-25**	**26-32**	**33-38**	**39-45**	**46-53**	**Average Growth Rate**
Algae Only (Control)	3	4.0 ± 1.1	-0.30 ± 1.0^{a}	0.05 ± 0.8^{ab}	1.7 ± 1.1^{a}	-	-	-	-	1.1 ± 3.0^{a}
Probiotic Only (Control)	4	1.3 ± 1.1	3.2 ± 1.9^{b}	-0.30 ± 1.3^{b}	1.2 ± 0.9^{a}	1.8 ± 1.2^{b}	0.50 ± 0.7^{b}	-	-	1.1 ± 2.4^{a}
Low	4	2.2 ± 2.1	3.4 ± 1.5^{b}	2.9 ± 0.7^{a}	4.4 ± 2.3^{ab}	4.6 ± 0.6^{a}	6.0 ± 1.4^{a}	3.2 ± 1.4	4.5 ± 2.3	3.3 ± 2.1^{b}
Med	4	1.6 ± 1.4	7.4 ± 2.1^{c}	2.0 ± 1.8^{ab}	3.4 ± 1.3^{ab}	2.3 ± 0.6^{ab}	4.1 ± 1.9^{ab}	4.7 ± 1.7	2.8 ± 3.5	3.1 ± 2.8^{b}
High	4	-0.04 ± 1.9	8.0 ± 0.4^{c}	2.6 ± 1.9^{ab}	5.9 ± 1.4^{b}	3.3 ± 1.7^{ab}	3.0 ± 3.2^{ab}	3.9 ± 2.0	3.0 ± 1.7	3.0 ± 2.9^{b}
Group Pr(>F)		0.061	**<0.001**	**0.022**	**0.004**	**0.020**	**0.018**	0.501	0.636	**<0.001**

Table 2.7. Water quality analysis for Experiment 2 (mean ± SD). Means followed by a common letter are not significantly different ($p<0.05$). Analysis method indicates the use of ANOVA (A) or Kruskal Wallis (K) and Tukey's Post Hoc (T) or Dunns Test (D), when appropriate. Bold indicates significant group differences ($p<0.05$) and star (*) indicates no significant difference found in post-hoc pairs analysis. SPC=Specific Conductivity.

Treatment Name	**n**	**Temp (°C)**	**DO (mg/L)**	**pH**	**SPC (µS/cm)**	**Ammonia (NH_3 mg/L)**	**Ammonium (NH_4 mg/L)**
Algae Only (Control)	19	22.6 ± 1.17	9.16 ± 0.42^{b}	7.48 ± 0.36	157.0 ± 3.00^{a}	0.000 ± 0.000	0.093 ± 0.020^{a}
B1000	26	23.1 ± 0.98	7.86 ± 0.42^{d}	7.62 ± 0.35	250.0 ± 35.5^{c}	0.003 ± 0.005	0.158 ± 0.280^{c}
B1002	19	22.9 ± 1.05	8.46 ± 0.53^{c}	7.57 ± 0.25	174.0 ± 24.2^{b}	0.000 ± 0.000	0.097 ± 0.019^{a}
B1008	24	22.8 ± 1.23	8.92 ± 0.41^{ab}	7.41 ± 0.33	155.0 ± 10.9^{a}	0.001 ± 0.002	0.121 ± 0.021^{bc}
N1100-50x	19	22.6 ± 1.22	8.90 ± 0.38^{abc}	7.52 ± 0.32	155.0 ± 2.18^{a}	0.000 ± 0.000	0.089 ± 0.190^{a}
NiteOut	26	22.7 ± 1.18	8.71 ± 0.34^{acd}	7.43 ± 0.32	156.0 ± 3.47^{a}	0.005 ± 0.002	0.101 ± 0.024^{ab}
Glosso	20	22.5 ± 0.93	8.67 ± 0.62^{acd}	7.51 ± 0.22	155.0 ± 2.19^{a}	0.001 ± 0.002	0.111 ± 0.013^{b}
Analysis Method		K	A/T	A	K/D	K/D	A/T
Group Pr(>F)		0.620	**<0.001**	0.208	**<0.001**	**0.017(*)**	**<0.001**

Table 2.8. Observed survival (mean ± SD %) of Experiment 2 juvenile yellow lampmussel (*Lampsilis cariosa)*. Raw data averaged from four initial replicates per treatment (except B1002 which had three replicates). Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$).

		Day							
Treatment	**n**	**0**	**7**	**16**	**23**	**30**	**37**	**44**	**51**
Algae Only (Control)	4	100 ± 0	52.3 ± 17.9^{b}	22.1 ± 20.3^{b}	3.0 ± 3.9^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
B1000	4	100 ± 0	65.2 ± 22.6^{b}	37.9 ± 14.5^{b}	21.4 ± 10.1^{b}	17.1 ± 7.9^{b}	15.1 ± 7.1^{ab}	13.0 ± 6.4^{ab}	11.1 ± 5.2^{b}
B1002	3	100 ± 0	46.8 ± 16.3^{b}	10.8 ± 7.3^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
B1008	4	100 ± 0	3.15 ± 2.5^{a}	1.55 ± 0.8^{a}	0.75 ± 0.3^{a}	0.25 ± 0.4^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
N1100-50x	4	100 ± 0	57.5 ± 4.2^{b}	17.8 ± 3.9^{b}	3.6 ± 1.2^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
NiteOut	4	100 ± 0	42.2 ± 4.4^{b}	18.0 ± 4.4^{b}	7.05 ± 4.2^{ab}	5.7 ± 4.4^{ab}	2.4 ± 2.4^{a}	1.7 ± 1.6^{a}	0.9 ± 1.1^{a}
Glosso	4	100 ± 0	43.1 ± 14.4^{b}	11.1 ± 9.5^{a}	1.9 ± 2.2^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Group Pr(>F)			**<0.001**	**<0.006**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Table 2.9. Logistic regression analysis results of survival data for Experiment 2 juvenile yellow lampmussel (*Lampsilis cariosa).* Odds ratio indicate change in survival odds (in percent) compared to the reference (intercept) group.

Treatment	**Log Odds**	**Odds Ratio**	**SE (Odds Ratio)**	**2.5%-97.5% Confident Interval (Odds Ratio)**	**Odds Ratio (Percent Change)**	**z-value**	**Pr>χ^2**
Algae Only (Control)- Intercept	2.52	12.50	0.729	11.14-14.01		43.20	<0.001
B1000	-1.10	0.33	0.023	0.29-0.38		-16.05	<0.001
B1002	0.26	1.30	0.128	1.07-1.58		2.61	0.009
B1008	0.53	1.70	0.207	1.34-2.17		4.30	<0.001
N1100-50X	0.20	1.22	0.104	1.03-1.44		2.31	0.021
NiteOut	-1.00	0.40	0.028	0.33-0.44		-13.41	<0.001
Glosso	-0.50	0.95	0.081	0.81-1.27		-0.56	0.575
Time- Intercept	-0.26	0.77	0.004	0.76-0.77	-23.00	-53.57	<0.001
B1000*Time	0.18	1.19	0.006	1.18-1.20	19.00	34.00	<0.001
B1002*Time	-0.90	0.91	0.009	0.90-0.93	-9.00	-8.60	<0.001
B1008*Time	-0.50	0.62	0.012	0.59-0.64	-38.00	-25.12	<0.001
N1100-50X*Time	-0.02	0.98	0.007	0.97-1.0	-2.00	-2.26	0.024
NiteOut*Time	0.09	1.10	0.006	1.09-1.11	10.00	16.38	<0.001
Glosso*Time	-0.05	0.95	0.008	0.93-0.96	-5.00	-6.44	<0.001

Null deviance: 75708 on 215 degrees of freedom
Residual deviance:7645 on 202 degrees of freedom
AIC: 8256

Table 2.10 Mussel growth rates (mean ± SD µm/day) for Experiment 2 juvenile yellow lampmussel (*Lampsilis cariosa).* Raw data averaged from four initial replicates per treatment (except B1002 which had three replicates). Bold indicates significant group difference using one-way ANOVA ($p<0.05$). Means followed by a common letter are not significantly different (Tukeys post-hoc analysis, $p<0.05$). Due to mortality and loss of complete replicates resulted in B1008 (n=1) sampling day 30; and NiteOut (n=3) sampling days 44 and 51.

		Sampling Interval (days)							
Treatment	**n**	**0-7**	**8-16**	**17-23**	**24-30**	**31-37**	**38-44**	**45-51**	**Average Growth Rate**
Algae Only (Control)	4	5.4 ± 4.9	4.7 ± 4.1	3.3 ± 3.3	-	-	-	-	3.4 ± 3.1
B1000	4	5.8 ± 1.9	1.1 ± 1.8	5.6 ± 3.6	6.4 ± 1.0^{a}	10.5 ± 4.3	7.3 ± 2.1	8.0 ± 2.8	5.7 ± 2.2
B1002	3	5.4 ± 2.1	3.7 ± 0.38	-	-	-	-	-	3.0 ± 0.82
B1008	4	10.4 ± 2.4	2.4 ± 3.5	1.7 ± 3.6	-0.43^{b}	-	-	-	3.4 ± 2.2
N1100-50x	4	6.4 ± 2.4	3.0 ± 1.5	7.1 ± 2.1	-	-	-	-	4.1 ± 1.5
NiteOut	4	7.0 ± 2.3	5.8 ± 1.4	2.5 ± 2.6	4.5 ± 2.3^{ab}	5.6 ± 4.6	6.2 ± 6.1	6.4 ± 6.8	4.6 ± 3.0
Glosso	4	8.6 ± 1.4	4.5 ± 0.58	4.1 ± 1.6	-	-	-	-	4.3 ± 0.89
Group Pr(>F)		0.187	0.154	0.0636	**0.0374**	0.166	0.736	0.639	0.262

Table 2.11. Water quality analysis for Experiment 3 (mean ± SD). SPC=Specific Conductivity. Means followed by a common letter are not significantly different ($p<0.05$). Analysis method indicates the use of ANOVA (A) or Kruskal Wallis (K) and Tukey's Post Hoc (T) or Dunns Test (D), when appropriate. Bold indicates significant group differences ($p<0.05$). Treatment codes refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and algae (A).

Treatment Name	**n**	**Temp (°C)**	**DO (mg/L)**	**pH**	**SPC (µS/cm)**	**Ammonia (NH_3 mg/L)**	**Ammonium (NH_4 mg/L)**	**Total Ammonia Nitrogen ($NH_4 + NH_3$ mg/L)**
QAC	35	20.7 ± 0.56	7.37 ± 0.85^{a}	7.59 ± 0.14^{bc}	149 ± 2.31^{abcd}	0.004 ± 0.005^{b}	0.285 ± 0.081^{bc}	0.289 ± 0.085^{bc}
LAC	33	20.7 ± 0.58	7.24 ± 0.0.88^{a}	7.59 ± 0.16^{bc}	158 ± 6.31^{bcd}	0.005 ± 0.006^{b}	0.294 ± 0.086^{bc}	0.298 ± 0.089^{bc}
QBA	33	20.7 ± 0.58	7.66 ± 0.88$^{a\,b}$	7.67 ± 0.14^{ab}	178 ± 3.52^{e}	0.007 ± 0.006^{b}	0.340 ± 0.088^{b}	0.346 ± 0.092^{b}
LBA	31	20.8 ± 0.60	7.87 ± 0.85b	7.71 ± 0.18^{a}	200 ± 14.1^{ef}	0.008 ± 0.005^{b}	0.332 ± 0.080^{b}	0.340 ± 0.083^{b}
QNA	32	20.6 ± 0.57	7.90 ± 0.90b	7.54 ± 0.13^{c}	152 ± 2.83^{abcd}	0.007 ± 0.006^{b}	0.437 ± 0.142^{a}	0.443 ± 0.147^{a}
LNA	34	20.7 ± 0.58	7.94 ± 0.84b	7.60 ±0.18^{ac}	166 ± 18.8^{cde}	0.011 ± 0.007^{a}	0.581 ± 0.188^{a}	0.592 ± 0.193^{a}
QN	21	20.6 ± 0.47	7.78 ± 1.17b	7.48 ±0.16^{c}	142 ± 0.87^{abcd}	0.003 ± 0.004^{b}	0.325 ± 0.070^{b}	0.328 ± 0.076^{b}
LBNA	23	20.6 ± 0.53	7.69 ± 0.97^{a} b	7.67 ±0.18^{ac}	190 ± 16.2^{ef}	0.010 ± 0.008^{a}	0.492 ± 0.144^{a}	0.501 ± 0.149^{a}
Analysis Method		T	K/D	T/A	K/D	K/D	K/D	K/D
Group Pr(>F)		0.694	**<0.001**	**0.034**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Table 2.12. Observed survival (mean ± SD %) for Experiment 3 juvenile yellow lampmussel (*Lampsilis cariosa).* Raw data averaged from replicates. Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$). Treatment codes refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and algae (A)

		Day					
Treatment	**n**	**0**	**7**	**14**	**21**	**28**	**35**
LAC	3	100 ± 0	79.5 ± 4.6^{ab}	51.9 ± 8.5 $^{a\ b}$	36.1 ± 13.8 ^{a}	23.5 ± 5.1 ^{a}	21.7 ± 6.1^{a}
QAC	3	100 ± 0	68.4 ± 3.5^{b}	45.0 ± 11.6 ^{b}	30.5 ± 15.3 ^{a}	24.8 ± 11.0 ^{a}	22.1 ± 10.0^{a}
LNA	4	100 ± 0	87.8 ± 5.3^{a}	64.9 ± 6.8 ^{a}	32.6 ± 8.3 ^{a}	24.3 ± 7.8 ^{a}	18.9 ± 6.8^{a}
LBA	3	100 ± 0	21.5 ± 6.0^{d}	6.3 ± 3.6 ^{c}	3.2 ± 1.8 ^{b}	2.3 ± 1.3 ^{b}	2.5 ± 1.5^{b}
QBA	3	100 ± 0	46.4 ± 6.6^{c}	17.3 ± 2.3 ^{c}	5.7 ± 2.1 ^{b}	4.1 ± 1.6 ^{b}	3.9 ± 0.9^{b}
QNA	3	100 ± 0	78.0 ± 5.6^{ab}	51.6 ± 7.3 $^{a\ b}$	33.3 ± 2.2 ^{a}	27.2 ± 5.2 ^{a}	25.2 ± 5.9^{a}
QN	2	100 ± 0	47.8 ± 11.7^{c}	5.8 ± 5.3 ^{c}	0.5 ± 0.7 ^{b}	0 ± 0 ^{b}	0 ± 0 ^{b}
LBNA	4	100 ± 0	23.4 ± 8.5^{d}	4.7 ± 2.8 ^{c}	0.6 ± 1.0 ^{b}	0 ± 0 ^{b}	0 ± 0 ^{b}
Group Pr(>F)			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Table 2.13. Logistic regression analysis results of survival data for Experiment 3 juvenile yellow lampmussel (*Lampsilis cariosa).* Odds ratio indicate change in survival odds (in percent) compared to the reference (intercept) group. Treatment codes refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and algae (A).

Treatment	**Log Odds**	**Odds Ratio (*e*)**	**SE (Odds Ratio)**	**2.5%-97.5% Confident Interval (Odds Ratio)**	**Odds Ratio (Percent Change)**	**z-value**	**Pr>χ^2**
LAC - Intercept	4.47	87.66	121	5.89-1292.49		3.26	0.001
QAC	-0.44	0.64	0.068	0.52-0.79		-4.23	<0.001
LBA	-0.97	0.38	0.046	0.30-0.48		-7.95	<0.001
LNA	0.50	1.65	0.184	1.33-2.06		4.50	<0.001
QBA	-0.49	0.61	0.073	0.49-0.77		-4.10	<0.001
QNA	-0.31	0.73	0.08	0.59-0.91		-2.90	0.004
QN	1.22	3.39	0.827	2.14-5.57		5.00	<0.001
LBNA	0.40	1.49	0.252	1.07-2.09		2.60	0.018
Time	-0.11	0.89	0.003	0.89-0.90	-11.00	-29.86	<0.001
QAC*Time	0.01	1.01	0.005	1.00-1.02	1.00	1.60	0.111
LBA*Time	-0.13	0.88	0.008	0.86-0.90	-12.00	-13.54	<0.001
LNA*Time	-0.30	0.97	0.005	0.96-0.98	-3.00	-5.29	<0.001
QBA*Time	-0.10	0.91	0.007	0.89-0.92	-9.00	-12.60	<0.001
QNA*Time	0.01	1.01	0.005	1.00-1.02	1.00	1.26	0.207
QN*Time	-0.35	0.71	0.02	0.67-0.74	-29.00	-12.39	<0.001
LBNA*Time	-0.34	0.71	0.014	0.68-0.74	-29.00	-18.03	<0.001

Null deviance: 24570 on 149 degrees of freedom
Residual deviance: 3002 on 134 degrees of freedom
AIC:3602

Table 2.14. Mussel growth rate (mean ± SD µm/day) for Experiment 3 yellow lampmussel (*Lampsilis cariosa)*. Raw data averaged from replicates per treatment. Bold indicates significant group difference using one-way ANOVA ($p<0.05$) and asterisk (*) indicates no significant difference found in post-hoc pairs analysis. Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$). Treatment codes refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and algae (A)

		Sampling Interval (days)					
Treatment	**n**	**0-7**	**8-14**	**15-21**	**22-28**	**29-35**	**Average Growth Rate**
LAC	3	0.5 ±1.9	10.5 ± 1.5^{a}	8.1 ± 2.4	10.4 ± 1.7^{ab}	6.4 ± 1.9	6.0 ± 1.6^{a}
QAC	3	4.2 ± 0.72	7.4 ± 1.1^{ab}	10.5 ± 5.9	-0.54 ± 3.6^{c}	7.0 ± 0.37	4.8 ± 1.9^{ab}
LNA	4	3.5 ± 3.2	4.3 ± 3.0^{ab}	3.7 ± 2.0	1.6 ± 1.0^{bc}	0.01 ± 2.7	2.2 ± 2.0^{ab}
LBA	3	-0.82 ± 1.4	7.3 ± 2.3^{ab}	7.1 ± 5.1	12.2 ± 5.1^{a}	-0.26 ± 5.1	4.3 ± 3.2^{ab}
QNA	3	2.2 ± 2.0	6.0 ± 2.7^{ab}	6.8 ± 1.5	-1.3 ± 1.6^{c}	4.1 ± 1.9	3.0 ± 1.6^{ab}
QBA	3	3.4 ± 2.0	5.0 ± 1.8^{ab}	6.5 ± 4.3	5.4 ± 6.5^{abc}	5.6 ± 6.5	4.3 ± 3.5^{ab}
QN	2	-1.5 ± 0.40	1.0 ± 0.14^{b}	-	-	-	-0.17 ± 0.18^{b}
LBNA	4	0.0 ± 1.2	3.5 ± 3.4^{b}	-	-	-	1.2 ± 1.5^{b}
Group Pr(>F)		**0.012(*)**	**0.012**	0.366	**0.002**	0.086	**0.007**

Figure 2.1. Primary rearing system design. Recirculating water system. Water flows from the feed bucket (below) into the rearing chamber (pictured right), discharging into the white PVC tubes and traveling back to the feed bucket. Photo: Virginia Martell

Figure 2.2. Rearing chamber design. Opaque tube is inflow of water solution and feed mixture and clear tube is discharge leading to common PVC discharge pipe. Water depth was approximately 2 inches and mesh size was 150-µm. Photo: Virginia Martell

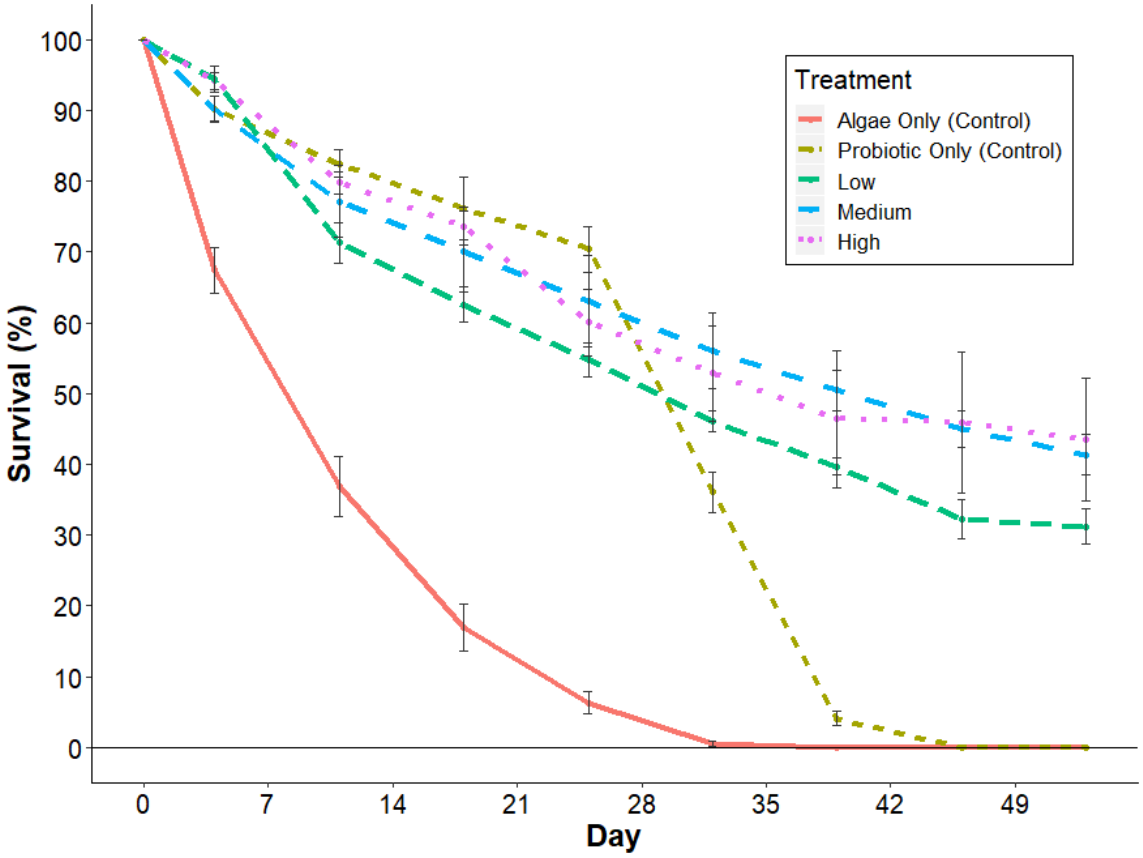

Figure 2.3. Observed survival (mean ± SD) of juvenile yellow lampmussel (*Lampsilis cariosa)* for Experiment 1 data averaged from four replicates (n) per treatment (except Algae Only (Control) which had three replicates) per sampling date.

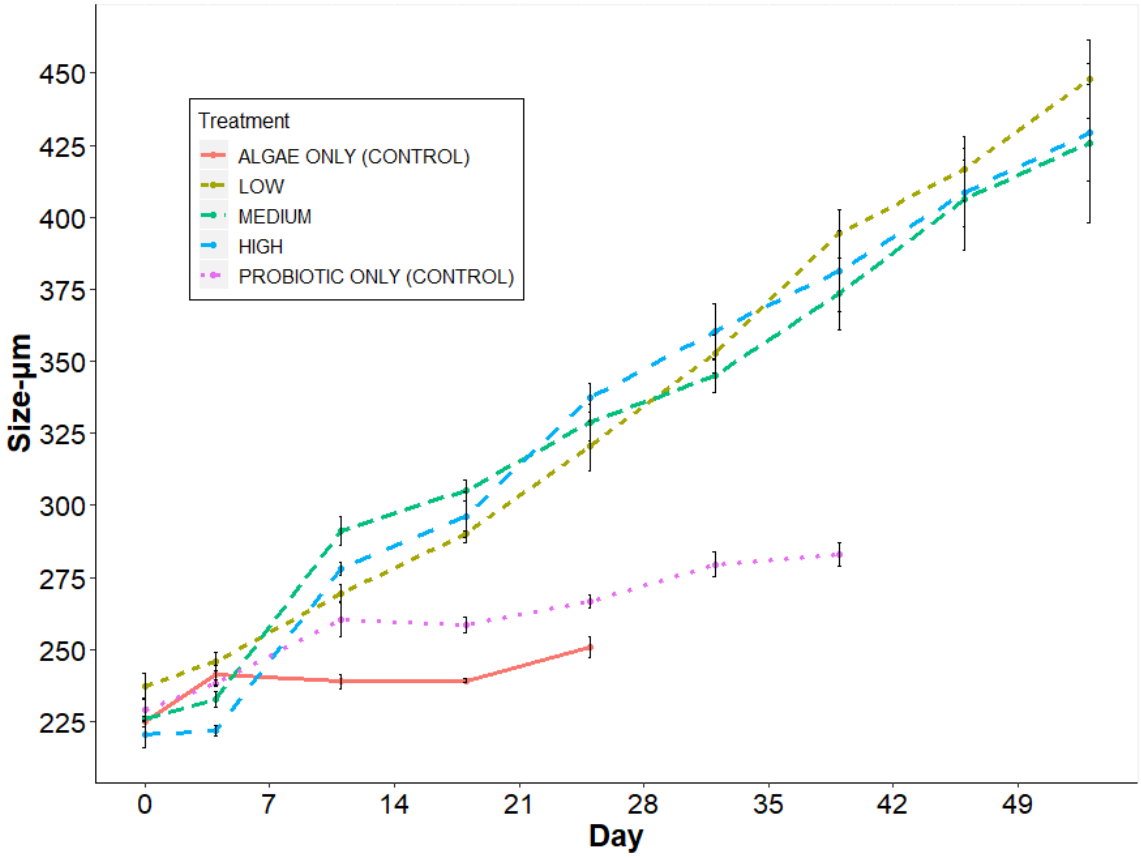

Figure 2.4. Average mussel size (mean ± SE) juvenile yellow lampmussel (*Lampsilis cariosa)* for Experiment 1 data averaged from four replicates (n) per treatment (except Algae Only (Control) which had three replicates) per sampling date.

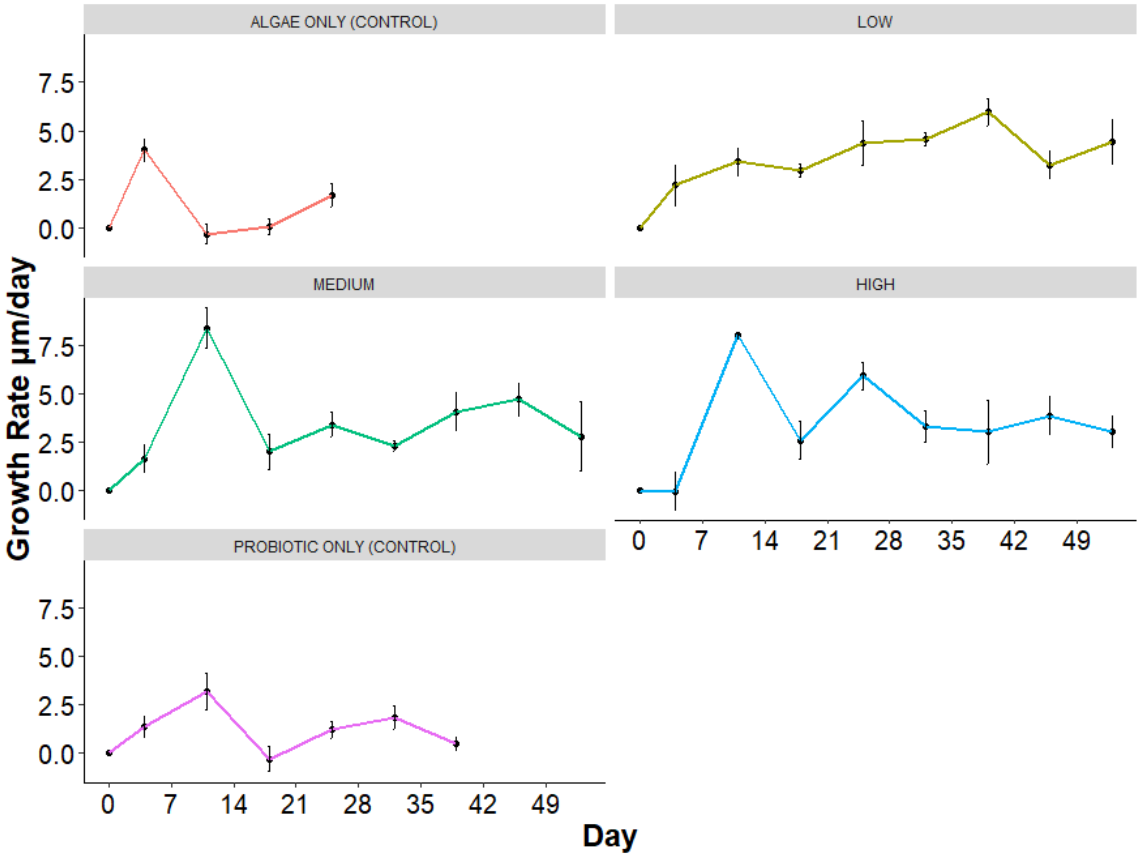

Figure 2.5. Observed mussel growth rates (mean ± SE) of juvenile yellow lampmussel (*Lampsilis cariosa)* for Experiment 1 data averaged from four replicates (n) per treatment (except Algae Only (Control) which had three replicates) per sampling date. Growth rates are plotted on the last day of the sampling interval (e.g. calculated growth rate between sampling day 0 and 4 is plotted on day 4).

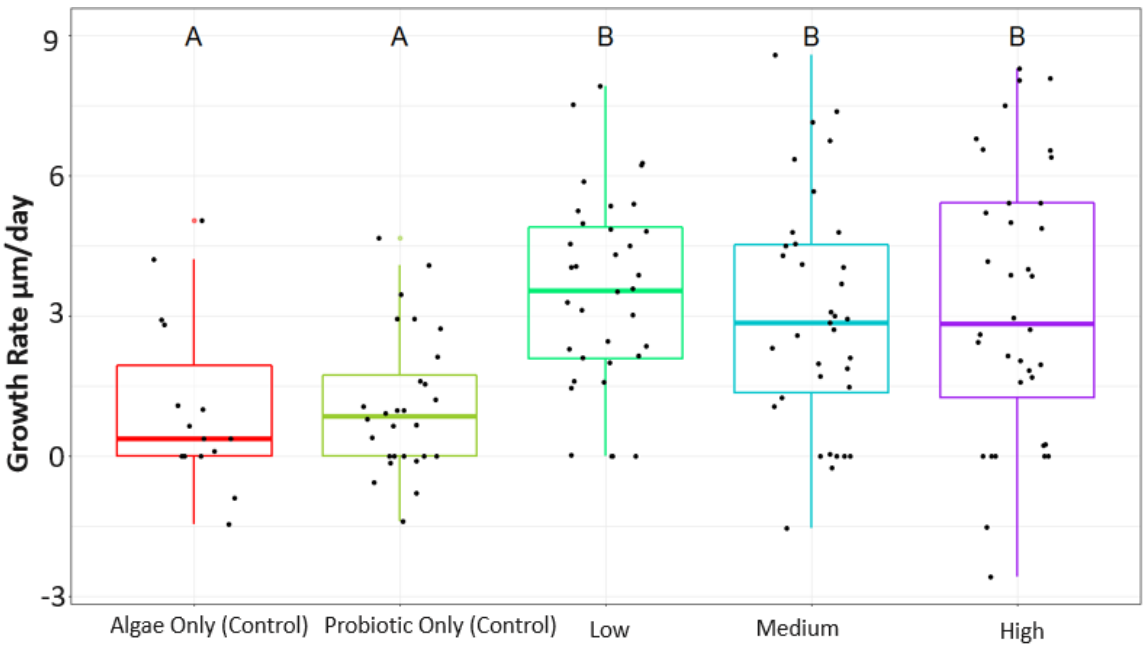

Figure 2.6. Observed range of juvenile yellow lampmussel (*Lampsilis cariosa)* growth rates for Experiment 1 data averaged from four replicates (n) per treatment (except Algae Only (Control) which had three replicates) per sampling date. Dots represent calculated growth rate for one sampling interval from averaged replicates.

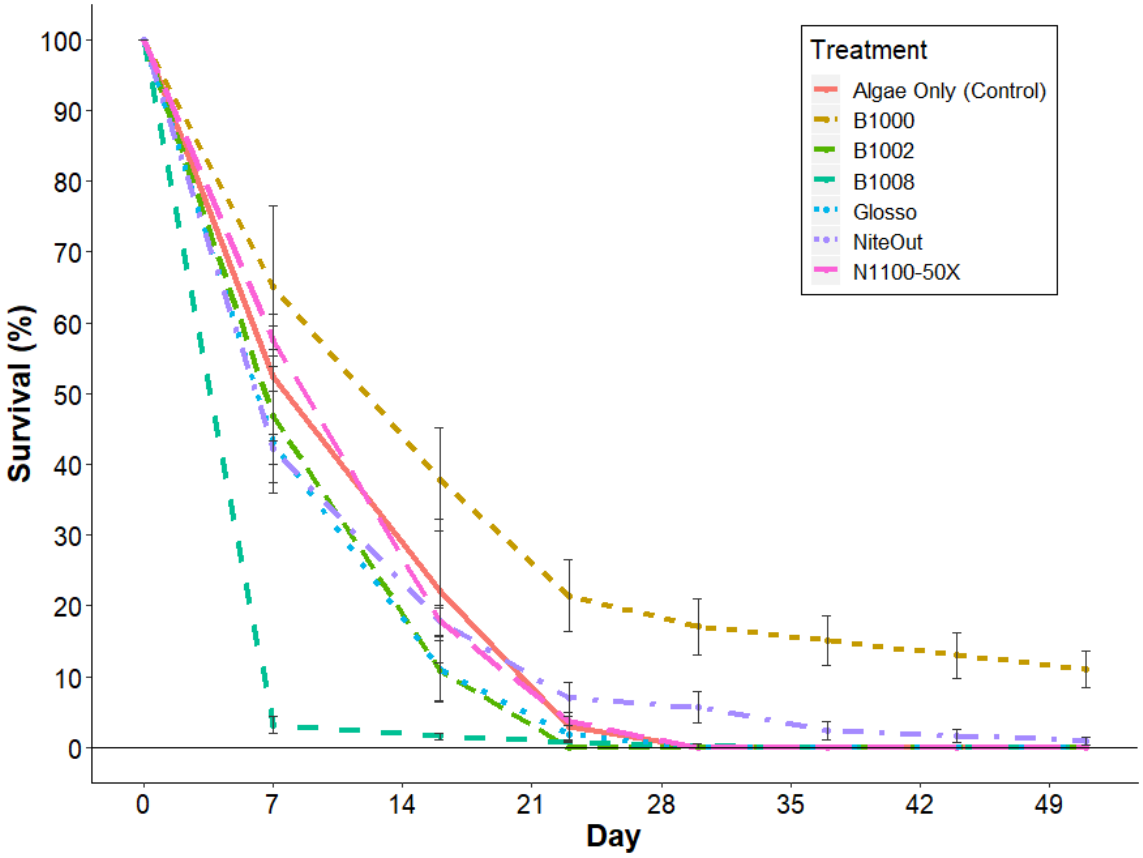

Figure 2.7. Observed survival (mean ± SD) juvenile yellow lampmussels *(Lampsilis cariosa)* for Experiment 2 data averaged from four initial replicates per treatment (except B1002 which had three replicates). Due to mortality and loss of complete replicates there were fewer replicates in B1008 (n=1) for day 30 and NiteOut (n=3) for days 44 and 51.

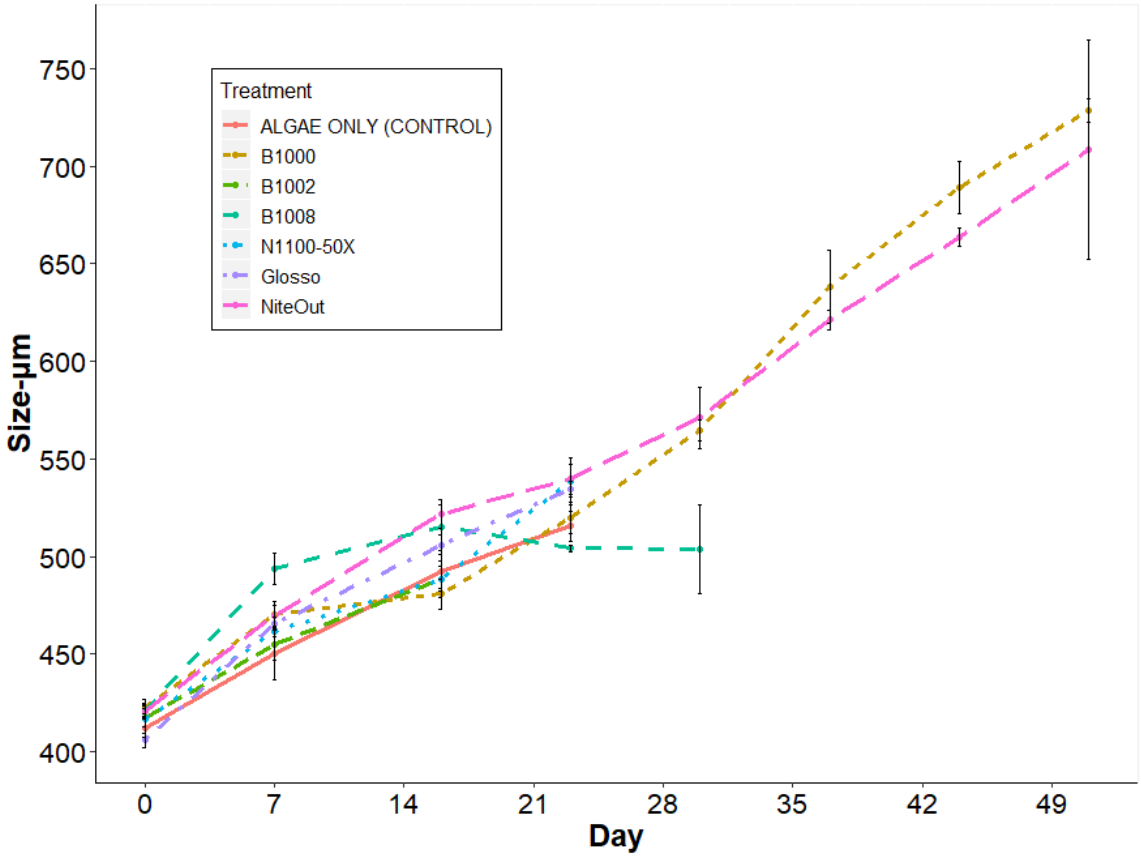

Figure 2.8. Average mussel size (mean ±SE) for juvenile yellow lampmussel (*Lampsilis cariosa)* for Experiment 2 based on four initial replicates per treatment (except B1002 which had three replicates) per sampling date. Mortality and loss of complete replicates resulted in fewer replicates for B1008 (n=1 on sampling day 30) and NiteOut (n=3 on sampling days 44 and 51).

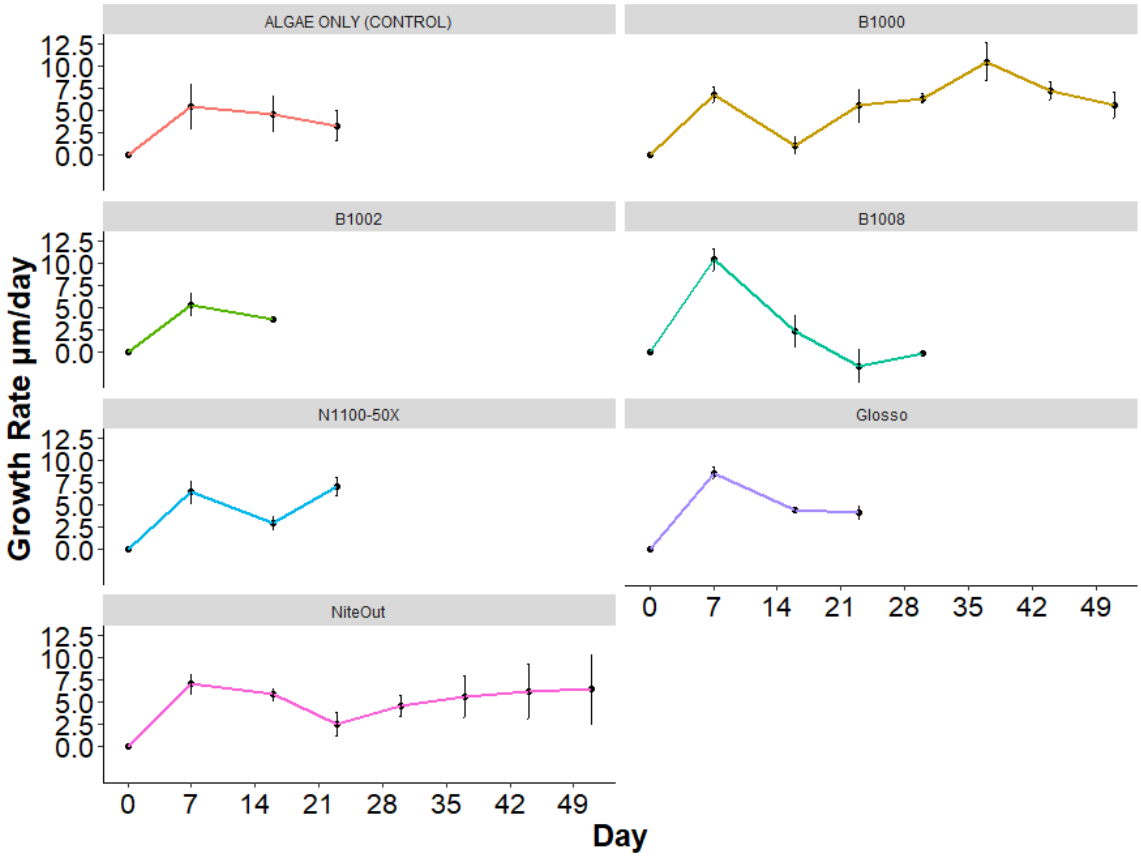

Figure 2.9. 14 Average mussel growth rates (mean ± SE) of juvenile yellow lampmussel (*Lampsilis cariosa)* for Experiment 2. Raw data averaged from four initial replicates per treatment (except B1002 which had three replicates) per sampling date. Growth rates are plotted on the last day of the sampling interval (e.g. calculated growth rate between sampling day 0 and 7 is plotted on day 7). Mortality and loss of complete replicates resulted fewer replicates in B1008 (n=1) on day 30 and NiteOut (n=3) on days 44 and 51.

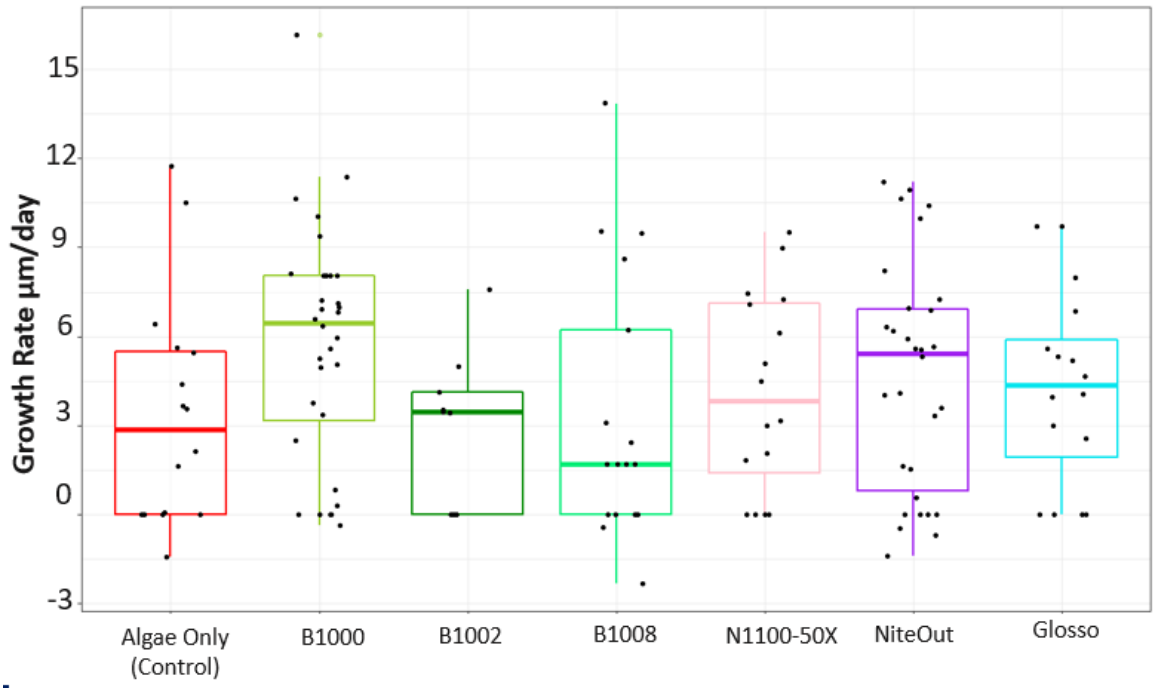

Figure 2.10. Observed range of juvenile yellow lampmussel (*Lampsilis cariosa)* growth rates for Experiment 2 data averaged from four replicates (n) per treatment (except Algae Only (Control) which had three replicates) per sampling date. Dots represent calculated growth rate for one sampling interval from averaged replicates. Due to mortality there were fewer replicates in B1008 (n=1) sampling day 30 and NiteOut (n=3) sampling days 44 and 51.

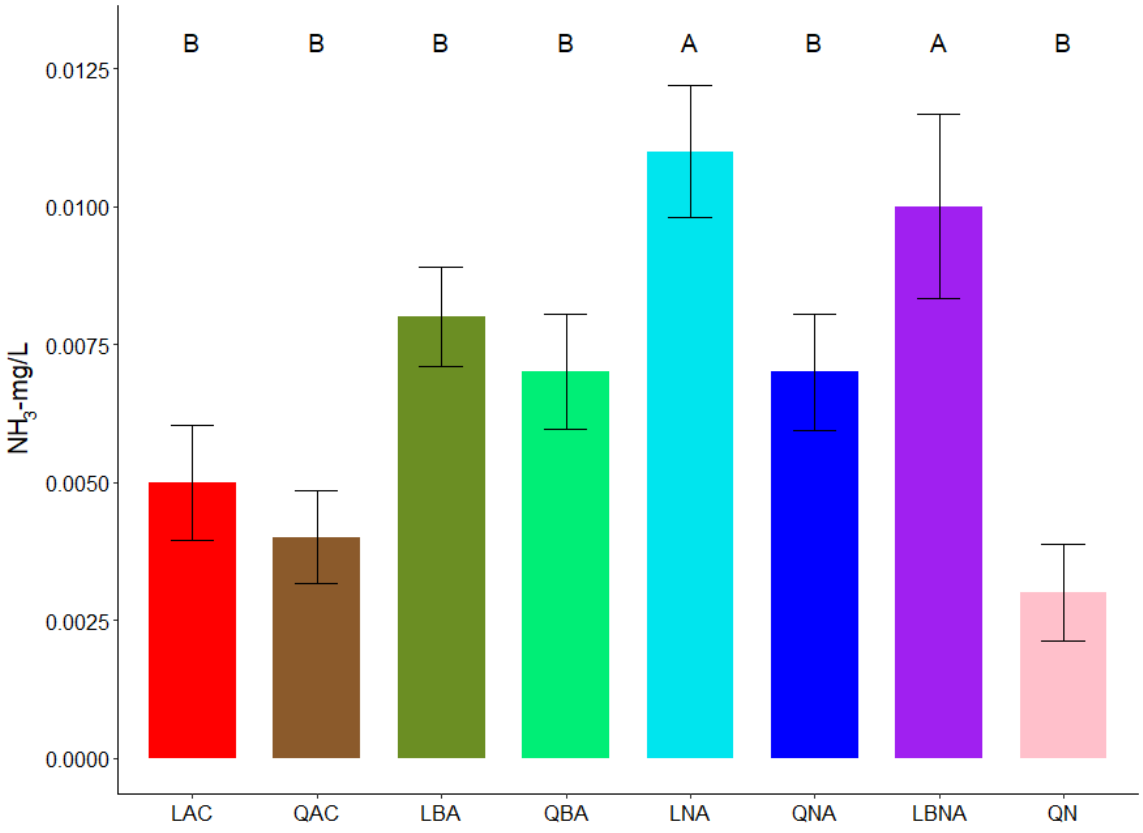

Figure 2.11. Average (mean ± SD) ammonia (NH_3-N) concentration among treatments for Experiment 2. Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$).

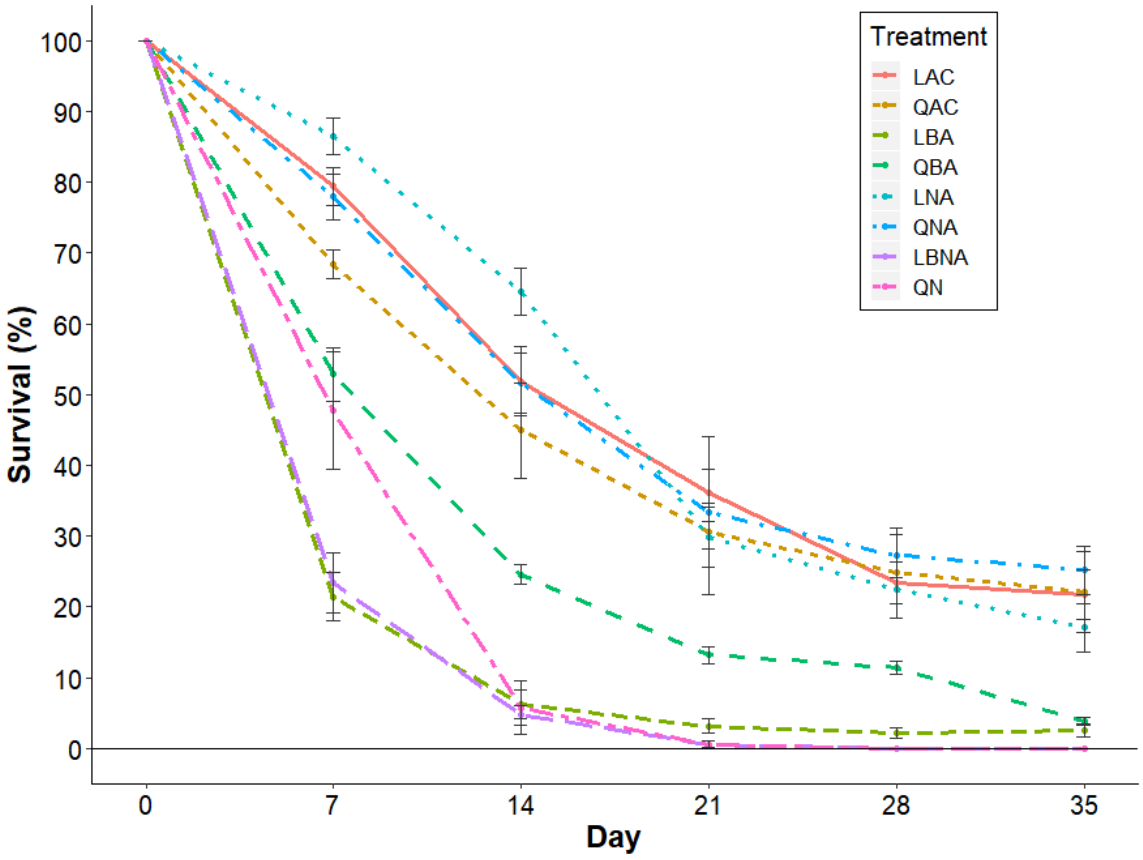

Figure 2.12. Observed survival (mean ± SD) of juvenile yellow lampmussels *(Lampsilis cariosa)* for Experiment 3. Raw data averaged from initial replicates (n=4) LNA, LBNA; (n=3) LAC, QAC, LBA, QBA, QNA; (n=2) QN per sampling date. Treatment codes in text refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and addition of algae (A).

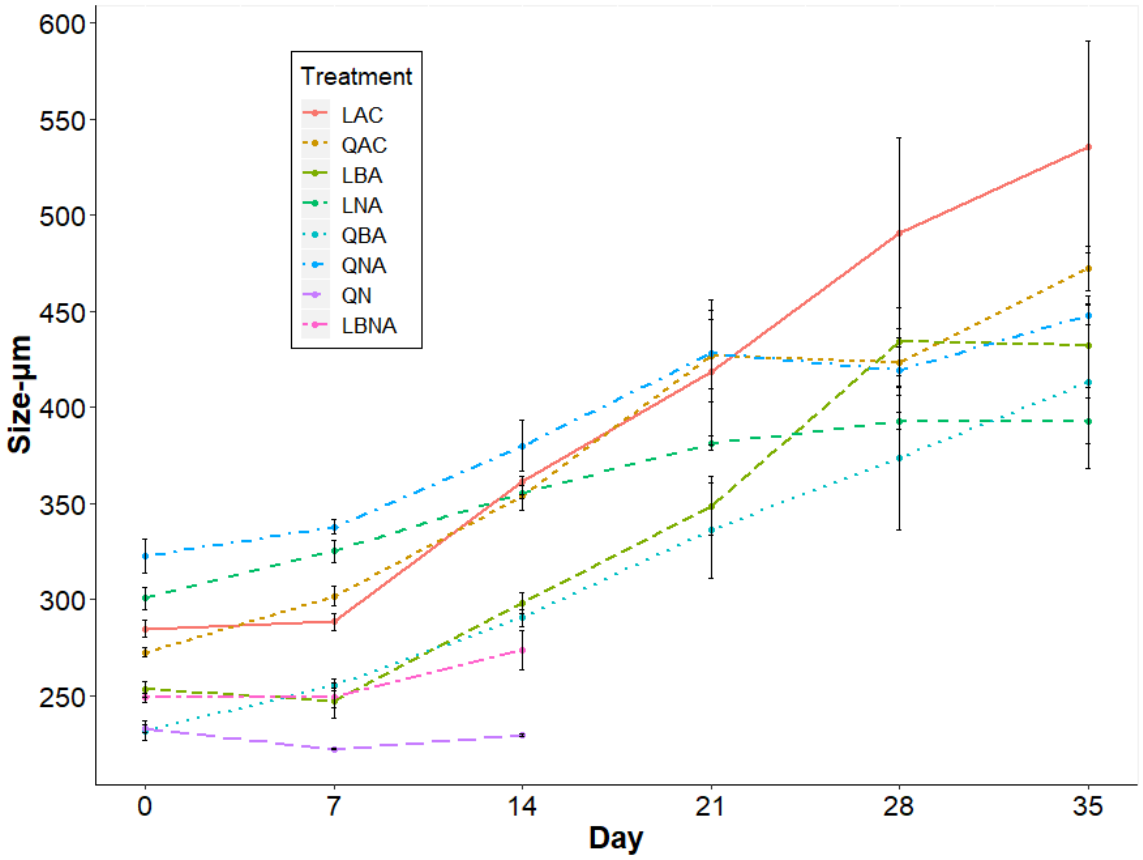

Figure 2.13. Average mussel size (mean ± SE) juvenile yellow lampmussel (*Lampsilis cariosa)* for Experiment 3. Raw data averaged from initial replicates (n=4) LNA, LBNA; (n=3) LAC, QAC, LBA, QBA, QNA; (n=2) QN. Treatment codes refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and algae (A).

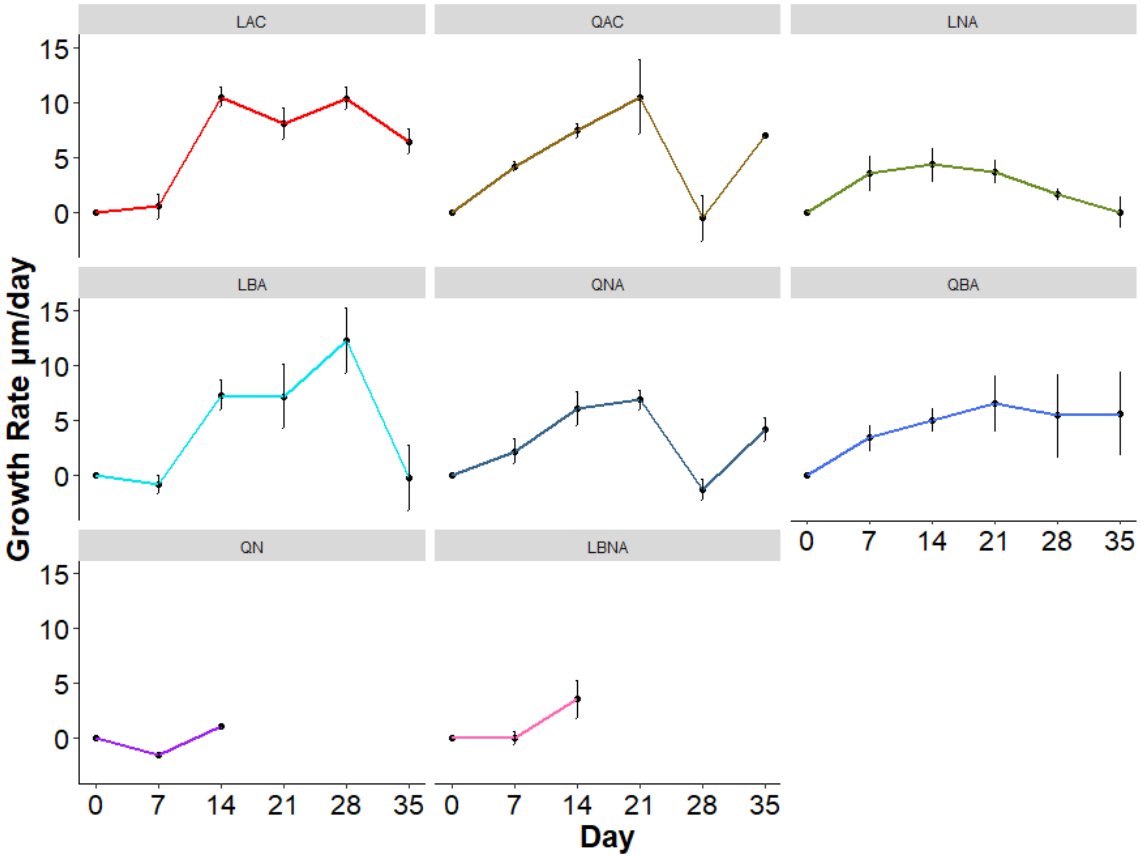

Figure 2.14. Average mussel growth rates (mean ± SE) of juvenile yellow lampmussel (*Lampsilis cariosa)* for Experiment 3. Raw data averaged from initial replicates (n=4) LNA, LBNA; (n=3) LAC, QAC, LBA, QBA, QNA; (n=2) QN. Treatment codes refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and algae (A). Growth rates are plotted on the last day of the sampling interval (e.g. calculated growth rate between sampling day 0 and 7 is plotted on day 7).

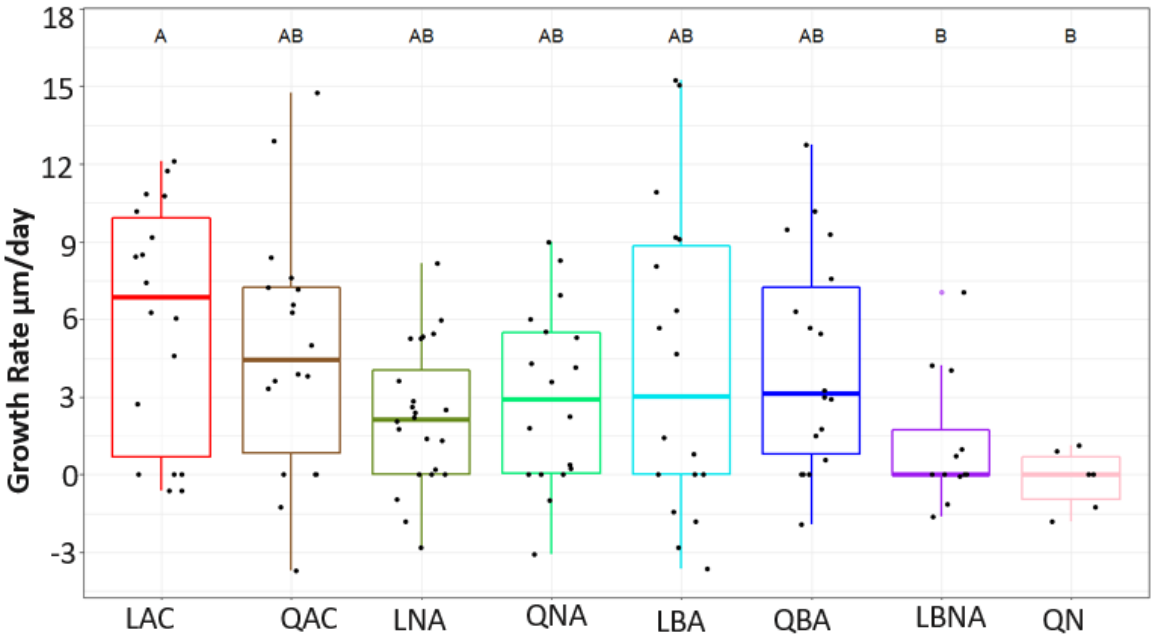

Figure 2.15. Average mussel growth rates (mean ± SE) juvenile yellow lampmussel (*Lampsilis cariosa)* for Experiment 3. Raw data averaged from initial replicates (n=4) LNA, LBNA; (n=3) LAC, QAC, LBA, QBA, QNA; (n=2) QN. Treatment codes refer to frequency of water change-out (Q = quick, L = long), addition of probiotic (B = *Bacillus*, N = NiteOut), and algae (A). Dots represent calculated growth rate for one sampling interval from averaged replicates.

CHAPTER 3
GROWTH AND SURVIVAL OF JUVENILE EASTERN LAMPMUSSELS IN SECONDARY REARING SYSTEMS

3.1 Introduction

North America is a global hotspot for freshwater mussels (family Unionidae) with over 250 species present (Lopes-Lima 2017); however, 65% of mussel species are endangered, threatened, or vulnerable (Haag and Williams, 2013). Anthropogenic factors such as habitat degradation and land use change are major contributors to freshwater mussel declines (Haag, 2012, Lopes-Lima 2017). Recently, propagation and culturing of freshwater mussels has been reinvigorated for the purpose of restoring populations through reintroduction or augmentation; at least 18 facilities in the United States have developed freshwater mussel programs over the last two and a half decades (Patterson et al. 2018). In 2015, the Richard Cronin Aquatic Resource Center (CARC) in Sunderland, MA began research as the first mussel propagation facility in New England.

The development of freshwater mussel programs has led to substantial advancements in culture; and improvement of culturing techniques has increased juvenile release to native systems in the last two decades (Zale and Neves 1982, Zimmerman and Neves 2002, Neves 2004, VADGIF 2010). Because mussels are highly vulnerable after metamorphosis, culturing practices of mussels is typically categorized by juvenile mussel size. Primary culturing of juvenile mussels consists of newly metamorphosed and young juvenile mussels up to 5 mm in length (or when growth rates slow), and secondary culture is considered larger (>3–5 mm) juvenile mussels (Patterson et al., 2018). Captive mussels that are released at a larger size experience increased survival and higher capture probability than mussels that are smaller in size (Meador et al. 2011); therefore, studies

comparing rearing systems are essential to develop optimal strategies to produce individuals that are of releasable size.

Many rearing systems are used for mussel culture, including: floating baskets (Mummert 2001), upwelling systems (Mair 2013), downwelling systems (Barnhart et al. 2006, Mair 2013), dogpans (Mair 2013), aquaria (Zimmerman 2003, Kotitvahdi et al. 2008), sand trays (Yang 1996), simulated stream channels (Beaty 1999), troughs, cages (Buddensiek 1995, Gatenby 2000, Brady et al. 2011), nets (Gatenby 2000), bunkers, and silos (Patterson et al. 2018). Most of these systems may be adapted for use in indoor facilities using flow-thru or recirculating water (Dunn and Layzer 1997, O'Beirn 1998, Henley et al. 2001, Fobian et al. 2015) or outdoors, such as in hatchery raceways, ponds, or rivers (Buddensiek 1995, Yang 1996, Hanlon 2000, Mummert 2001, Zimmerman 2004). Differences in secondary rearing systems such as flow rate, the presence of sediment and sediment size (Beaty and Neves 2004, Jones et al. 2005, Liberty et al. 2007), presence of predator control (Zimmerman et al. 2003), food type and amount (Gatenby et al 1996, 1997, 2003; Hua et al. 2013, Mair 2013), ammonia, (Augspurger et al. 2003), and temperature (Buddensiek 1995, Beaty and Neves 2004, Carey et al. 2013) impact growth and survival of cultured juvenile mussels. Because environmental factors differ among rearing locations, rearing systems that work in one facility may not work at another facility (Patterson et al. 2018). Furthermore, freshwater mussels are highly diverse with differing environmental or habitat requirements among species; as such, effectiveness of rearing systems varies among species (Yang 1996, Gatenby 2000, Mummert 2001, Mair et al. 2013, Patterson et al. 2018). Thus, facility-specific and species-specific secondary rearing methods are needed.

The goal of this study was to investigate the effect of different secondary rearing systems on the growth and survival of eastern lampmussel (*Lampsilis radiata*). I tested several culturing systems that were indoors (dogpans and baskets) or outdoors (trough, airlift upweller, tank upweller, baskets) and compared rearing systems located in two New England locations: U.S. Fish and Wildlife Service Richard Cronin Aquatic Resource Center (CARC, Sunderland, Massachusetts), and U.S. Fish and Wildlife Service North Attleboro National Fish Hatchery (NANFH, North Attleboro, Massachusetts). To test the effect of mussel size on survival and growth, I compared two different size classes of juvenile mussels (> 5.0 mm and <5.0 mm) using the same culture system (trough) at two different outdoor culture locations. I predicted that the mussels within the trough and baskets would have higher growth compared to other treatments due higher quality of natural food resources and constant water flow that mimics natural river flow, and that the dogpans would have the lowest average growth because they are fed a commercial algae diet and have a reduced ability to flush excess nutrient build-up. This study will provide information on secondary culture for yellow lampmussel in New England.

3.2 Methods

3.2.1 Study Location and Design

Both study locations are adjacent to wild water rivers and ponds. The water source for CARC is a mixture of surface water from two spring-fed ponds and hatchery effluent water that combine and flow into a concrete fish raceway. The upper raceway is divided into 3 sections, each 30 m x 4 m. A partially recirculating system was created by pumping water from the bottom of the first raceway section through a large black water

tube to a fiberglass flow-thru retaining tank at the top of the first raceway. The water was then distributed to the hatchery building via a pump, as needed. One rearing system was located indoors while all other rearing systems were located outdoors at the raceway. CARC is located in temperate central-western Massachusetts and is part of the Connecticut River watershed. Summer temperatures (July-September) average 30–34°C and winters (December-February) average -12–1°C.

NANFH is primarily a shad and brook trout hatchery. The water source for the hatchery and shad ponds is diverted from Bungay River located upstream of the hatchery. Upon discharge from the hatchery or shad pools, the water is diverted into a series of effluent ponds downstream of the hatchery where the pond baskets rearing systems were located. Two rearing systems were located inside the hatchery, one rearing system was located outside within a tented fish pool, and all other systems were located outdoors either in the effluent pond or within a reach of the Bungay River. NANFH is located in eastern Massachusetts and is approximately 50 km west of Massachusetts Bay and 24 km north of the innermost section of Narragansett Bay. NANFH in part of the Ten Mile River watershed. Average summer and winter temperatures are similar to those at CARC.

3.2.2 Study Species

The eastern lampmussel (*Lampsilis radiata*) is found throughout the Atlantic Slope from the north of Nunavut, Canada through Ontario, Quebec, New Brunswick, and Nova Scotia south to South Carolina, United States. Eastern lampmussel is a common species throughout all of New England, except Rhode Island where it is critically imperiled (NatureServe 2019). Eastern lampmussels are regarded as generalist occurring in streams, large rivers, and lakes with both slow-moving and fast-moving currents; in sandy, rocky,

or muddy substrate, slow-moving or fast-moving currents. They are a medium sized species reaching a maximum total length of 127 mm and they can live up to 40 years (Nedeau 2008).

The eastern lampmussel was used in this study as a surrogate species for yellow lampmussel (*L. cariosa)*, which is listed as endangered in Massachusetts, Connecticut, and Delaware; and is threatened or imperiled in 5 other states (see Chapter 1). The eastern lampmussel is similar to the yellow lampmussel in several physiological characteristics such as size and fecundity. Both species are found in medium sized rivers with sandy substrates, including in the Connecticut River watershed (Nedeau 2008) and they have similar host-fish species. The eastern lampmussel (*Lampsilis radiata)* has been used a surrogate species for the yellow lampmussel previously (Kurth et al. 2017), and thus was deemed appropriate in this study to minimize use of the state-endangered species.

3.2.3 Rearing System Designs

The study included 5 different rearing systems: trough, floating baskets, dogpans, airlift upweller, and tank upweller, which are described in detail below. At CARC the dogpans were located indoors and the airlift upweller, tank upweller, and troughs were located outdoors at the raceways. At NANFH the dogpans and baskets were in the fish hatchery building, the airlift upweller was located within an interior fish pool, and the trough and baskets were located outdoors. Treatment specifics, such as location (indoor vs. outdoor, CARC vs. NANFH), water source, filtration type, flow type (flow-thru or recirculating), flow rate, sediment use and size, and food source are in Table 1.

Each rearing system had 3 replicates of 120 juvenile mussels (age 8–10 months, size 5–8 mm, see Appendix C), except the CARC tank upweller (4 replicates), and NANFH airlift upweller (2 replicates). The troughs had two size class of mussels, ([trough-1 (small), average 4 mm, age 8-10 months; and trough-2 (large), size 5–8 mm, age8-10 months)] in both locations. The experiment lasted 56 days from the end of July through the end of September 2017.

3.2.3.1 Trough

The troughs were 3.5 m x 0.4 m x 0.2 m (length x width x height) metal containers placed downstream of a valve-controlled water outflow from a natural water source. At CARC the trough was placed within the concrete fish raceway and the water source was the effluent pond upstream of the raceway. At NANFH the trough was placed below a damned section of the Bungay River. Three 22 m x 15 m x 5 cm baskets for each mussel size class were placed in the trough. The bottom of each basket was lined with 1x1-mm mesh, the sides were enclosed by 5 x 3-mm metal mesh, and the top remained open.

3.2.3.2 Floating basket

Floating baskets (described in Patterson et al. 2018) were used at the NANFH. Floating baskets (22-cm diameter) were placed in both an indoor waterway (hatchery basket, n=3) and an outdoor pond (pond basket, n=3). Each plastic mesh basket was lined with 1-mm mesh on all sides. A 145-cm long foam noodle was wrapped around the top rim of the basket and attached using zip ties. In the pond, the three baskets were anchored

to a line for deployment and retrieval. In the indoor waterway, all 3 baskets were anchored together and placed in a concrete water raceway.

3.2.3.3 Dogpan

Dogpans (described in Patterson et al. 2018) were used at both CARC and NANFH. Three 27 x 10.5 cm circular plastic culture pans were mounted above a drainage sump with a 1.25-cm bulkhead and 9.5 x 2.5-cm standpipe in the center of the pan. At CARC, the dogpans used water pumped from the wild water retaining tank that was filtered through a 300-µm mesh screen inside the hatchery. The wild water entered the dogpan via a pump-manifold line located at the top of the pan, exited through the standpipe, and returned to the drainage sump which was then recirculated back through the system. Wild water was acquired from an exterior pond that was a mix of surface water and groundwater. The water was sterilized with a UV light and filtered through 5-µm mesh. The CARC dogpans were supplemented with algae feed during the study. The dogpan sump was changed out once a week, and 2.0 mL of Marine Microalgae Concentrates™, 2.0 mL of Shellfish Diet 1800™, and 1.0 mL of Nanno 3600™ (Reed Mariculture Inc, Campbell, CA) was added to the water. An additional 1.0 mL of Marine Microalgae Concentrates, 1.5 mL of Shellfish Diet, and 0.75 mL of Nanno diet was administered three days after water change-outs.

At NANFH water to the indoor hatchery system was filtered through a 50-µm sand filter and UV sterilization system. The dogpans were on a flow-through water system with filtered water entering via a pump-manifold line located at the top of the pan and exiting through the standpipe to the drainage sump that discharged from the facility

3.2.3.4 Airlift upweller

The airlift upweller was a modified version of the Suspended Upweller System (SUPSY) described in Patterson et al. (2018). The airlift upwellers were used at both CARC and NANFH. The airlift upwellers were constructed of two nested 3.7-L buckets both with layer of 1.5-mm and 10-mm mesh for bottoms, a 25 x 2.5 cm polyvinyl chloride (PVC) tube airlift, an air pump and air valve, and a brick. A PVC tube was inserted into the top bucket lid and an air pump line was connected to the PVC tube via an air valve. Instead of the floating at the water surface (as with SUPSY), the buckets were tied to a brick and sunk to the bottom of the water source. The top of the PVC tube sat 10-12 cm above the surface of the water. The 1.5-mm mesh screen that served as the bottom of the airlift upweller acted as a coarse filter. At CARC, the airlift upwellers were sunk to the bottom of a large fiberglass flow-thru wild water retaining tank that was outside located next to the raceways. At NANFH the Airlift upwellers were placed sunk to the bottom of a large concrete flow-thru fish pool that was inside fish culture building. The pool water was filtered through a 50-µm, 1/8 plastic bead media filter and UV sterilization system prior to entering the system. Because the airlift tube forces water through the top, water is forced to upwell through the rearing chamber.

3.2.3.5 Tank upweller

The flow-thru tank upweller system located at CARC was a modified version of the tank upwellers described in Patterson et al. (2018). The tank upweller system was constructed of two 98-L circular main tanks that each held two 3.7-L buckets for holding mussels. The buckets were lined with layer of 1.5-mm and 10-mm mesh to prevent mussel escapement and debris buildup within the chambers. A T-shaped drain was

constructed of 5.0-cm PVC and used to connect the two culturing chambers. Water from the wild water retaining tank was pumped through a 300-µm mesh screen into the Tank upweller system, then a second pump was used to fill the circular main tanks. As water entered from the sump to the main tanks, it upwelled through the bottom of the buckets and was then discharged through the T-shaped drain back to the sump. A drain line was attached to the sump to discharge water back to the raceway creating a partial flow-thru system.

3.2.4 Water Quality

Water quality was measured at each sampling period, approximately every 14 days. The measurements were taken from the source water of the rearing system (e.g. in the retaining tank, circular tanks, trough, pond, shad pool, and concrete raceway). Because small and large mussels were located within the same trough, one measurement was used for both systems. Dissolved oxygen (DO) and was measured using a YSI™ ProODO Optical Dissolved Oxygen Meter (YSI Inc/Xylem Inc, Yellow Springs, OH). Salinity, total dissolved solids (TDS), specific conductivity (SPC), and pH were measured using a LaMotte™ Tracer Pocketester (LaMotte Company, Chestertown, MD). Ammonium (NH_4-N), calcium Carbonate ($CaCO_3$), and chloride (Cl-) were tested using a YSI™ 9300 photometer (YSI Inc/Xylem Inc, Yellow Springs, OH). Temperature was measured every 4 h throughout the experiment with an Onset HOBO® Pro v2 Data Logger (Onset Computer Corporation, Boston, MA).

3.2.5 Mussel Measurements (Survival and Growth)

All mussels were photographed on the first day of the experiment and every 14 days thereafter. Contents of rearing chambers rinsed through 1-, 0.90-, and 0.50-µm mesh to separate debris from mussels. Mussels were then transferred to petri dishes and photographed using an EOS 5DSR camera (Canon USA Inc, Huntington, NY). Multiple petri dishes were used per replicate, as needed. Debris, such as sticks or algae build-up, and dead mussel shells were removed from the dish prior to taking a picture.

All mussels were enumerated from pictures and a subset were measured for estimating growth rates. Using Microsoft Excel® a simple random sample was conducted to select grid cells for juvenile measurement. Juveniles that were lying completely flat with >50% body area within the selected cell boundaries were measured posterior to anterior end to the nearest one-hundredth mm using Image Pro Insight software (Media Cybernetics, Rockville, MD). Mussels within additional randomly selected grid cells were measured until 25 mussels per replicate were measured. An *a priori* power-analysis for ANOVA determined that a sample size of 25 mussels per replicate would have a power of 0.99 to detect an effect size of 0.27 with a significance of 0.05 (R pwr package version 1.2–2).

3.2.6 Data Analysis

All data were assessed for model assumptions (i.e., residuals, homogeneity of variance, normality, deviance, etc.). Non-parametric alternatives were used if assumptions were violated. In all analyses, the rearing system was a categorical factor. Days and water quality variables were continuous variables. R statistical software

(version 3.4.4; R Core Team 2018) was used to calculate all statistics. All statistical tests were evaluated at the α=0.05 significance.

3.2.6.1 Water Quality

To test for differences in water quality among treatments by location and between treatments and locations, water quality parameters (temperature, DO, pH, Cl- $CaCO_3$, NH_4-N, salinity, TDS, SPC) were analyzed using a one-way analysis of variance (ANOVA) and Tukey's post-hoc analysis. If assumptions of normality were not met, non-parametric Kruskal-Wallis and Dunn's rank sum multiple comparison test with Bonferroni correction were used (car package version 3.0-2 and dunn.test package version 1.3.5).

3.2.6.2 Survival

To test the difference in odds of survival among treatments, a binomial logistic regression model (link=logit) with maximum likelihood estimation was used (R stats package version 3.4.4). The dogpans were used as the reference for interpretation. Treatment, time, and location were evaluated as significant predictors of survival odds (denotated as odds ratio=OR). For the binomial logistic regression model, Hosmer & Lemeshow goodness-of-fit (ResourceSelection package 0.3-5), likelihood ratio test (lmtest package 0.9-36), and pseudo-R^2 values (rcompanion package version 2.2.1) were used to assess fit. Predicted survival values were inspected (R stats package version 3.4.4). Final survival percentage at day 56 was compared using a one-way ANOVA. To test the difference in observed survival of rearing systems between locations, and to test

the difference in size between trough systems, Welch's two sample t-test for unequal variance was used (R stats package version 3.4.4).

3.2.6.3 Growth

Growth rates were calculated for each replicate and sampling interval as:

$$\frac{\text{shell length at sampling time}^{i} - \text{ shell length at sample time}^{i-1}}{\text{time}^{i} - \text{time}^{i-1}}$$

Growth rates were compared among rearing systems using one-way ANOVA and Tukey's post-hoc analysis (car package version 3.0-2).

3.3 Results

3.3.1 Rearing System Comparisons

At NANFH, one replicate from the airlift upweller was removed from analysis due to mussel escapement of >50% of the population prior to the first sampling period. For day 56, one replicate of the pond baskets was removed due escapement of >50% of the mussels. Mussel escapement was not directly observed in other rearing systems; however, survival counts indicate that if escapement was happening, it was relatively minor.

3.3.1.2 Water Quality

There were significant differences in all water quality parameters among rearing systems. The highest average temperature was 25.5 ± 1.1°C in the CARC dogpans, and the lowest average temperature was 19.2 ± 2.2 °C in the CARC trough system (Table 3.2, Appendix H). The trough at NANFH had the lowest DO concentration (7.55 ± 0.87

mg/L) while the NANFH pond baskets had the highest DO (9.86 ± 1.44 mg/L). The CARC dogpans had the highest pH (7.40 ± 0.18) and the NANFH airlift upwellers had the lowest pH (7.09 ± 0.04). TDS, salinity, and SPC were all lowest in the CARC airlift upwellers; and were all highest in the NANFH pond baskets rearing systems. NH_4-N was the highest in the CARC dogpans and lowest in the NANFH trough. Calcium carbonate levels was generally the lowest in the CARC tank upweller system (11.2 ± 17.6) and significantly higher in the NANFH pond baskets (44.8±7.2 mg/L). Levels of chloride in the NANFH pond baskets (7.42 ± 4.03 mg/L) were the highest among rearing systems; and levels in the NANFH airlift upwellers were the lowest (1.20 ± 1.39 mg/L).

3.3.1.3 Survival

Observed mussel survival across rearing systems using mussels greater than 5.0 mm was not significantly different (p=0.0825) and ranged from 70.0% (NANFH hatchery baskets) survival to 96.8% (CARC dogpan) survival (Figure 3.1, Table 3.3). Model comparison for survival among rearing systems indicates that treatment, time, and location had a significant effect on odds of survival; therefore, I compared combinations of rearing systems and location. Time decreased the odds of survival by 5%. The odds ratio (OR) indicates that all treatments odds of survival were significantly lower than the CARC dogpans except the CARC airlift upweller (p=0.643). Odds of survival for juvenile mussels decreased by 86% for NANFH airlift upweller, 81% for NANFH hatchery baskets, 76% for the NANFH dogpans, 75% for NANFH trough (large size class), 69% for CARC tank upweller, 60% for NANFH pond baskets, 56% for CARC trough (large size class), 8% for the CARC airlift upweller (Table 3.4).

3.3.1.4 Growth

ANOVA indicates no significant differences in average sizes among rearing systems at day 0: CARC trough-2 (5.69 ± 0.11), pond baskets (5.61 ±0.12), CARC dogpan (5.60 ± 0.17), CARC tank upweller (5.48 ± 0.10), NANFH airlift upweller (5.47 ±0.08), NANFH trough-2 (5.47 ± 0.02), NANFH dogpan (5.46 ±0.21), NANFH hatchery baskets (5.34 ± 0.11), and CARC airlift upweller (5.18 ± 0.70) (p=0.389). After 56 days mussel sizes were significantly different between rearing systems with the larger size class of mussels: NANFH pond baskets (14.24 ± 0.39), CARC airlift upwellers (10.38 ± 0.54), CARC tank upweller (10.00 ± 0.51), CARC trough (9.90 ± 0.70), CARC dogpan (8.69 ± 0.12), NANFH airlift upweller (7.06 ± 0.04), NANFH trough (6.00 ± 0.05), NANFH dogpan (5.83 ± 0.14), NANFH hatchery baskets (5.65 ± 0.09) (Figure 3.2, Appendix F). Average growth rates of the 56 day study were significantly higher in the NANFH-pond baskets treatments (0.156 ± 0.081 mm/day) than all other treatments except the CARC airlift upweller (0.076 ± 0.098 mm/day) and CARC tank upweller (0.074 ± 0.110 mm/day) (Table 3.5. Figure 3.3, Figure 3.4). The NANFH hatchery baskets had the lowest average growth rates (0.006 ± 0.21 mm/day).

Significant differences in week-to-week growth rates indicate the NANFH pond baskets had consistently higher growth rates at each sampling interval, except at sampling interval 14-28 where the NANFH pond baskets, CARC airlift upweller, CARC tank upweller, and CARC trough had similar growth rates. The highest growth rates of all treatments (except NANFH trough) occurred between days 14 and 28. Specifically, the CARC airlift upweller, CARC trough (large size class), CARC tank upweller, and NANFH pond baskets growth rates increased by approximately 250-700% between

sampling days 14 and 28. Week-to-week growth rates remained fairly consistent for the NANFH dogpans, NANFH hatchery baskets, and NANFH trough (large size class), with no significant increases compared to the previous week.

3.3.2 Paired Comparison of Treatments (CARC v. NANFH)

Survival of mussels in the trough small size class was significantly lower at NANFH (10.4 ±7.4) compared to CARC (48.1±7.1) ($p=0.003$); however, there was no significant difference in total survival between locations for the dogpans, airlift upweller, and trough with larger size class mussels (Table 3.6, Figure 3.5). For all treatments, average size at day 56 was higher at CARC (Table 3.7, Figure 3.6). There were significant differences in growth rates between locations for the dogpan and troughs (both size classes), with all growth rates being higher at CARC (Table 3.11, Figure 3.7).

3.3.3 Size Comparison

At CARC and NANFH two different size classes of juvenile mussels (small v. large) were compared using the trough rearing system. At CARC, survival of mussels was higher for the large mussels (90%) compared to the small mussels (48%) (Table 3.9, Figure 3.8). Similarly, at NANFH survival of the large mussels (76%) was significantly higher than survival of the small mussels (10%, Figure 3.10). Average growth rates at CARC were significantly higher for large mussels (0.065 ± 0.076) compared to the small mussels (0.016 ± 0.014, Table 3.10, Figure 3.9); however, at NANFH average growth rates were similar for the small (0.016 ± 0.014) and large (0.011 ± 0.020, Figure 3.11) mussels.

3.4 Discussion

Eastern lampmussel survival and growth varied based on the type and location of different secondary rearing systems, with CARC dogpans having the highest survival and NANFH pond baskets with the highest growth rates. There were also significant differences in survival between size classes, with the larger size class having significantly better survival, suggesting that when deploying juvenile mussels into outdoor culture systems, mussels size should be greater than 5.0 mm. Growth rates were similar in the two size classes at NANFH, but at CARC the larger mussels had higher growth rates than smaller mussels.

3.4.1 Does secondary rearing system affect growth or survival of mussels?

There was no difference in average survival (range 70.0–98.6%) when average mussel size is greater than 5.0 mm, regardless of whether the rearing system was located at CARC or NANFH, indoors or outdoors, or was a flow-through or recirculating water system. After 56 days, average percent survival for the CARC dogpans and tank upwellers were similar to those of Mair (2013); and survival of the NANFH dogpans (71.3%) was lower than those noted by Mair (2013) at rates of 90.1–100% across species in the dogpan treatment. Dogpans used by Mair (2013) were recirculating systems that maintained higher average water temperature and were supplemented with an algae diet, compared to the dogpans at NANFH, which had lower average temperature and possibly less food availability. Overall high percentage of survival of mussels was attributed to the size of the juveniles used; as mussels larger than 5.0 mm are relatively robust (Mair 2013).

The floating baskets in the pond at NANFH had the highest growth rate of all other treatments. Higher production of algae and bacteria in the ponds compared to the river water (NANFH trough), effluent and surface water (CARC airlift upweller, CARC tank upweller, and CARC trough), and sterilized water (NANFH hatchery baskets, NANFH-dogpans, and NANFH airlift upwellers) may have contributed to increased growth rates. Natural "wild water" may provide benefits that sterilized or treated hatchery water does not, including higher abundances of food availability, supplemental nutrients, and vitamins (Gatenby et al. 1996). It has been suggested that bacteria may be a primary food source for freshwater mussels (Nicholas and Garling 2000), and that bacteria may enhance feeding efficiency and immune responses (WenYing et al. 2009, Bianchi et al. 2017). Filtration and UV sterilization would have significantly reduced the natural abundance and viability of bacteria in NANFH dogpans, NANFH hatchery baskets, and NANFH airlift upwellers (Liltved and Cripps 2001). Wild water has been shown to increase growth rates of mussels over rearing systems utilizing aquariums with cultured phytoplankton (Kovitvadhi et al 2006). Growth rates of *Lampsilis hagginsi* deployed into rivers were similar to those in our pond baskets (USFWS 2002, and Brady et al 2001).

Higher temperature in the pond baskets may have also attributed to significantly higher growth rates compared to other treatments. The pond is the seventh in a series of effluent discharge ponds downstream of the hatchery and maintained higher average temperatures than other rearing systems (except the CARC dogpans, Appendix I). Successful rearing of *Anodonta implicata* (alewife floater) had previous been demonstrated in this pond and was attributed to the warm temperatures and food availability (Hanlon, USFWS, pers.comm). Temperature has been shown to improve

growth rates in other *Lampsilis* species (Carey et al. 2013) and because temperature was significantly higher in the pond baskets than all other treatments this may have contributed to high growth rates in this study. For many freshwater mussel species, growth rates reflect patterns in temperature change at various life stages (Beaty 1999, Beaty and Neves 2004, Negishi and Kayaba 2010, Carey et al. 2013). In this study most treatments weekly average growth rates appeared to increase or decrease in response to weekly average temperature increase or decrease up to day 49; however, between day 49 and 56, growth rates did not increase in the CARC trough, airlift upweller, or tank upwellers when temperatures increased; and NANFH growth rates remained low (except in the pond baskets), similar to previous sampling periods (Appendix H). This decrease in average temperature from day 28 to day 44 and 49 may have decreased food availability and triggered physiological response of the mussels in preparation for colder temperatures in which growth slows and ceases (Doucet-Beaupre et al. 2010, Negishi and Kayaba 2010, Versteegh et al. 2010). The CARC dogpan growth rate mirrored temperature changes through day 56 and had the highest growth rate of all rearing systems during the final sampling period after day 44 (NANFH) and 49 (CARC), except the NANFH pond baskets. The CARC dogpans did not experience the same drastic decrease in temperature as the other treatments because the water was temperature controlled and it was the only system supplemented with algae feed.

Collectively, CARC dogpans, airlift upwellers, and troughs (both size classes) all maintained higher growth and survival than their NANFH counterparts, which could be explained by water quality, temperature, or food. There were several significant differences in water quality between CARC and NANFH treatments, but Salinity, SPC,

TDS, DO, NH_4-N, $CaCO_3$, Cl-, and pH were within normal range for rearing of freshwater mussels and would not be expected to influence survival or growth rates (Appendix H). Average temperature of the dogpans at CARC was significantly higher than the dogpans at NANFH, and these higher temperatures are likely contributed to higher growth. In addition, dogpans at CARC were supplemented twice weekly with algae, so the higher food availability at CARC may also explain the higher growth compared to NANFH. Temperature between the airlift upwellers and trough systems were similar between CARC and NANFH and food availability was not measured in this study, so it is unclear what contributed to higher growth at CARC vs. NANFH in these other systems.

Sediment within rearing systems may improve juvenile growth or survival of juveniles by enhancing the beneficial bacteria community and nutritional content (Hudson and Isom 1984, O'Beirn et al. 1998) or facilitating pedal feeding and food digestion (Gatenby et al. 1996, 1997). Similar growth and survival rates were seen between systems with sediment (dogpans, troughs) and systems without sediment (airlift upwellers, tank upwellers) at each location. Sediment may be critical to improve juvenile growth and survival at a young age, but may be less important for juveniles that no longer pedal-feed, or other differences between benefits may outweigh the positive effects of sediment use in the rearing systems.

3.4.2 Does size class of mussels affect growth and survival of mussels?

Size is a strong indicator of survival (Haag and Rypel 2010); however, the reason for this is unclear and may be due to a suite of variables. Mussels deployed at a smaller size class had significantly lower survival rates than all other treatments, despite having

similar water quality and food availability as the troughs with larger mussels. Mummert (2001) and Hanlon (2001) found that newly metamorphosed juveniles released into a fish raceway had little survival likely due to lower temperatures and inadequate nutrition in the water source related to seasonal phytoplankton and organic seston patterns. Smaller juveniles may possess fewer lipid reserves that can be accessed when food resources are limited, and smaller juveniles may feed selectively on smaller sized algae than larger individuals; therefore, the combined effect of mussel size, lower water temperatures, and decreased food quality and quantity due to seasonal flux may have reduced survival of the small size class mussels in the trough. Another explanation for reduced survival of the small size class is that at both locations, juvenile escapement from the trough system was apparent, with more escapement by smaller mussels than larger mussels, although escaped mussels were excluded from survival counts. Hanlon (2000) had similar problems with escapement due to the high motility of early juveniles when mussels are predominantly pedal feeding (Yeager et al. 1994, Gatenby et al 1996).

Similar average growth rates among small and large size classes at NANFH may be explained due to escapement or mortality of smaller individuals influencing growth rate calculations. I observed smaller empty shells during each sampling period; and the mortality of smaller individuals could potentially bias growth rate results indicating higher average growth rates (Barnhart et al. 2006).

3.5 Conclusions

Key factors to the success of freshwater mussel culture are preventing animal escapement, minimize mortality, and promoting growth. Holding conditions should meet mussel needs, including use of sediment for burrowing, food availability, nutritional

content, temperature regiment, ammonia load, and calcium requirements, which may differ across species and mussel developmental stage. Similar survival and growth were found among different rearing systems exposed to similar environmental conditions, suggesting that environmental variables are the driving factor in culture conditions, rather than specific aspects of the rearing system design.

Outdoor culture may be unsuitable for juvenile mussel sizes below 5.0 mm; however, the reason for this is unclear. An indoor secondary culture system for small mussels was not evaluated in this study, which may have exhibited similar growth and survival results as larger juvenile sizes (greater than 5.0 mm) under controlled conditions. Future studies should determine if mussels less than 5.0 mm can be moved to secondary culture systems under different environmental factors (e.g. more food availability, warmer average temperatures) to determine what variables contribute to mortality of juvenile mussels less than 5.0 mm in size.

The results of this study are pertinent to the culture of eastern lampmussel, but provide a broader context for survival and growth responses among several different commonly used secondary rearing systems. The relevance of the results to other species may depend on species-specific sensitivities to factors such as salinity, temperature, and nutritional content. The results of this study may apply to other mussel species, given environmental variables are considered during culture set-up. Natural habitat of mussels may provide information about essential environmental parameters for mussel culture. Ultimately, the results of this study may inform secondary culture of eastern lampmussel and related species, and advance culturing knowledge toward the ultimate goal of population restoration.

Table 3.1. Characteristics of secondary rearing systems used at Cronin Aquatic Resource Center (CARC) and North Attleboro National Fish Hatchery (NANFH). Trough-1 was the small size class and trough-2 was the large size class. UV = ultraviolet. N/A = not applicable.

Rearing system	Location	Indoor / Outdoor	Water Type	Primary Filtration	Flow	Flow Rate (L/min)	Sediment Size	Food Source
Dogpan	CARC	Indoor	Surface water / effluent	300-µm mesh screen	Recirculating	0.5	500≤1000-µm	Algae diet and wild water
Dogpan	NANFH	Indoor	Surface water	50-µm sand filter, UV sterilization	Flow-thru	0.5	500≤1000-µm	Wild water
Airlift upweller	CARC	Outdoor	Surface water / effluent	1.5-mm mesh	Flow-thru	1.5	N/A	Wild water
Airlift upweller	NANFH	Outdoor	Surface water	50-µm sand filter, 1/8" plastic bead media, UV sterilization	Flow-thru	1.5	N/A	Wild water
Trough-1	CARC	Outdoor	Surface water / effluent	Unfiltered	Flow-thru	6.0	500≤1000-µm	Wild water
Trough-1	NANFH	Outdoor	Surface water	Unfiltered	Flow-thru	6.0	500≤1000-µm	Wild water
Trough-2	CARC	Outdoor	Surface water / effluent	Unfiltered	Flow-thru	6.0	500≤1000-µm	Wild water
Trough-2	NANFH	Outdoor	Surface water	Unfiltered	Flow-thru	6.0	500≤1000-µm	Wild water
Basket-ponds	NANFH	Outdoor	Effluent	1.5-mm mesh screen	Flow-thru	13.0	≥1000- µm	Wild water
Basket hatchery	NANFH	Indoor	Surface water	50-µm sand filter, UV sterilization	Flow-thru	13.0	≥1000- µm	Wild water
Tank upweller	CARC	Outdoor	Surface water / effluent	300-µm mesh screen	Flow-thru	5.0	N/A	Wild water

Table 3.2. Water quality analysis for rearing systems (mean ± SD). Means followed by a common letter are not significantly different ($p<0.05$). Analysis method indicates the use of ANOVA and Tukey's Post Hoc (A/T) or Kruskal Wallis (KW) and Dunns Test (D). Bold indicates significant group differences ($p<0.05$) and an asterisk (*) indicates different sample sizes for analysis. For temperature n=56. TDS=Total Dissolved Solids. SPC= Specific Conductivity.

Rearing system	**Location**	**n**	**Temp (°C)***	**DO (mg/L)**	**pH**	**TDS (ppm)**	**Salinity (ppm)**	**SPC (µS/cm)**	**Ammonium (NH_4) (mg/L N)**	**$CaCO_3$ (mg/L)**	**Cl (mg/L)**
Dogpan	CARC	5	25.5 ± 1.1	8.27 ± 0.42	7.40 ± 0.18	102 ± 4	72 ± 4	156 ± 9	0.10 ± 0.06	15.8 ± 7.4	3.3 ± 2.6
Airlift Upweller	CARC	5	19.9 ± 2.3	7.67 ± 0.45	7.24 ± 0.08	58 ± 8	40 ± 0	100 ± 3	0.05 ± 0.04	16.8 ± 5.8	5.6 ± 2.0
Tank Upweller	CARC	5	22.3 ± 2.5	8.74 ± 0.53	7.31 ± 0.12	60 ± 10	44 ± 5	103 ± 6	0.05 ± 0.05	11.2 ± 17.6	3.7 ± 1.7
Trough	CARC	5	19.2 ± 2.2	7.98 ± 0.44	7.30 ± 0.24	56 ± 11	40 ± 0	102 ± 3	0.07 ± 0.09	13.4 ± 4.2	4.3 ± 3.3
Dogpan	NANFH	5	20.3 ± 1.8	8.65 ± 0.27	7.17 ± 0.07	362 ± 36	243 ± 27	537 ± 52	0.09 ± 0.09	39.8 ± 6.9	3.58 ± 1.35
Airlift Upweller	NANFH	5	19.6 ± 1.8	8.38 ± 0.58	7.09 ± 0.04	386 ± 46	256 ± 31	555 ± 64	0.04 ± 0.03	38 ± 8.8	1.20 ± 1.39
Trough	NANFH	5	19.9 ± 1.8	7.55 ± 0.87	7.26 ± 0.10	374 ± 36	254 ± 27	549 ± 53	0.02 ± 0.01	40.4 ± 16.6	2.00 ± 2.29
Baskets-Pond	NANFH	5	23.1 ± 1.8	9.86 ± 1.44	7.18 ± 0.13	430 ± 22	286 ± 24	611 ± 29	0.07 ± 0.02	44.8 ± 7.2	7.42 ± 4.03
Baskets-Hatchery	NANFH	5	19.9 ± 1.7	8.55 ± 0.31	7.15 ± 0.08	364 ± 31	244 ± 19	540 ± 48	0.08 ± 0.12	35.8 ± 5.8	6.74 ± 3.10
Analysis Method	NANFH		A/T	KW/D	KW/D	KW/D	KW/D	KW/D	KW/D	KW/D	A/T
Group Pr(>F)			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Table 3.3. Percent survival (mean ± SD) for eastern lampmussel (*Lampsilis radiata*) rearing systems averaged from replicates (n) per treatment. Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$). nd = no data

			Day					
Rearing System	**Location**	**n**	**0**	**14**	**28**	**44**	**49**	**56**
Dogpan	CARC	3	100	96.7 ± 1.3	96.3 ± 2.4	n.d	96.3 ± 3.1	96.8 ± 3.6[a]
Airlift Upweller	CARC	3	100	97.2 ± 4.8	96.2 ± 3.4	n.d	96.1 ± 4.0	94.1 ± 9.5[a]
Tank Upweller	CARC	4	100	92.5 ± 5.6	89.0 ± 7.9	n.d	88.0 ± 8.4	88.0 ± 10.5[a]
Trough-1	CARC	3	100	71.2 ± 4.5	57.5 ± 6.7	n.d	49 ± 5.2	48.1 ± 7.1[b]
Trough-2	CARC	3	100	93.0 ± 2.4	90.3 ± 7.0	n.d	89.3 ± 8.0	89.6 ± 10.4[a]
Dogpan	NANFH	3	100	93.7 ± 2.4	94 ± 3.0	89.5 ± 1.9	n.d	71.3 ± 15.7[a]
Airlift Upweller	NANFH	2	100	94.9 ± 2.3	81.8 ± 14.7	76.8 ± 20.6	n.d	70.5 ± 27.1[a]
Trough-1	NANFH	3	100	70.5 ± 11.3	43.8 ± 21.4	21.1 ± 9.7	n.d	10.4 ± 7.4[b]
Trough-2	NANFH	3	100	96.3 ± 1.7	91.6 ± 2.9	87.9 ± 5.4	n.d	75.9 ± 13.3[a]
Baskets-Pond	NANFH	3	100	95.8 ± 4.2	93.9 ± 6.4	89.7 ± 3.4	n.d	90.0 ± 4.71[a]
Baskets-Hatchery	NANFH	3	100	91.2 ± 1.8	90.6 ± 2.2	86.7 ± 6.6	n.d	70.0 ± 17.3[a]
Group Pr(>F)								<0.001

Table 3.4. Logistic regression analysis results for survival data eastern lampmussel (*Lampsilis radiata*) of rearing systems at CARC and NANFH. SE = standard error of odds ratio

Rearing system	**Location**	**Log Odds**	**Odds Ratio**	**SE (Odds Ratio)**	**2.5%-97.5% Confident Interval (Odds Ratio)**	**Odds Ratio (Percent Change)**	**z-value**	**Pr>χ^2**
Dogpan- Intercept	CARC	5.47	238	35.80	178.67-322.70	n/a	36.338	<0.001
Airlift Upweller	CARC	-0.09	0.915	0.18	0.63-1.33	-8.50	-0.46	0.643
Tank Upweller	CARC	-1.19	0.307	0.05	0.224-0.414	-69.30	-7.57	<0.001
Trough-1	CARC	-3.15	0.043	0.01	0.032-0.057	-95.70	-21.19	<0.001
Trough-2	CARC	-0.83	0.435	0.07	0.310-0.602	-56.50	-4.94	<0.001
Dogpan	NANFH	-1.44	0.236	0.04	0.171-0.321	-76.40	-9.07	<0.001
Airlift Upweller	NANFH	-1.95	0.143	0.02	0.103-0.195	-85.70	-12	<0.001
Trough-1	NANFH	-4.10	0.017	0.00	0.012-0.022	-98.30	-27.75	<0.001
Trough-2	NANFH	-1.39	0.249	0.04	0.183-0.334	-75.10	-9.04	<0.001
Baskets-Pond	NANFH	-1.34	0.262	0.04	0.189-0.356	-73.80	-8.34	<0.001
Baskets-Hatchery	NANFH	-1.67	0.19	0.03	0.190-0.356	-81.10	-10.64	<0.001
Time		-0.05	0.95	0	0.957-0.952	-5.00	-38.52	<0.001
Null deviance: 6595 on 169 degrees of freedom								
Residual deviance: 1541 on 158 degrees of freedom								
AIC: 2103								

Table 3.5. Mussel growth rate (mean ± SD mm/day) for eastern lampmussel (*Lampsilis radiata*) treatments averaged from replicates (n) per treatment. Bold indicates significant group difference using one-way ANOVA ($p<0.05$). Means followed by a common letter are not significantly different (Tukeys post-hoc analysis, $p<0.05$).

			Sampling Interval (days)						
Rearing System	**Location**	**n**	**0-14**	**14-28**	**28-44**	**28-49**	**44-56**	**49-56**	**Average Growth Rate**
Dogpan	CARC	3	0.070 ± 0.012	0.081 ± 0.014	nd	0.032 ± 0.016b	nd	0.046 ± 0.038	0.057 ± 0.028[b]
Airlift Upweller	CARC	3	0.026 ± 0.042	0.193 ± 0.015	nd	0.109 ± 0.030a	nd	-0.024 ± 0.097	0.076 ± 0.098a
Tank Upweller	CARC	4	0.058 ± 0.009	0.236 ± 0.049	nd	0.030 ± 0.016b	nd	-0.029 ± 0.061	0.074 ± 0.110a
Trough-1	CARC	3	0.023 ± 0.004	0.070 ± 0.001	nd	0.043 ± 0.008b	nd	0.012 ± 0.023	0.037 ± 0.025b
Trough-2	CARC	3	-0.009 ± 0.012	0.163 ± 0.015	nd	0.093 ± 0.012a	nd	0.013 ± 0.055	0.065 ± 0.076b
Dogpan	NANFH	3	0.011 ± 0.024	0.024 ± 0.008	-0.005 ± 0.012b	nd	-0.004 ± 0.015b	nd	0.007 ± 0.018b
Airlift Upweller	NANFH	2	0.038 ± 0.015	0.047 ± 0.004	0.001 ± 0.019b	nd	0.024 ± 0.021b	nd	0.027 ± 0.022b
Trough-1	NANFH	3	0.016 ± 0.011	0.023 ± 0.007	-0.001 ± 0.006b	nd	0.024 ± 0.017b	nd	0.016 ± 0.014b
Trough-2	NANFH	3	0.029 ± 0.01	0.002 ± 0.019	-0.010 ± 0.011b	nd	0.021 ± 0.009b	nd	0.011 ± 0.020b
Baskets-Pond	NANFH	3	0.103 ± 0.005	0.266 ± 0.061	0.104 ± 0.024a	nd	0.149 ± 0.02a	nd	0.156 ± 0.081a
Baskets-Hatchery	NANFH	3	0.004 ± 0.019b	0.016 ± 0.023	-0.003 ± 0.026b	nd	0.006 ± 0.022b	nd	0.006 ± 0.021b
Group Pr(>F)									<0.001

Table 3.6. Comparison of percent survival (mean ± SD) for CARC and NANFH treatments averaged from replicates (n) per treatment using Welch's two sample t-test for unequal variance.

	CARC	NANFH	Pr(<t)
Dogpan	96.8 ± 3.6 (3)	71.3 ± 15.7 (3)	0.100
Airlift Upweller	94.1 ± 9.5 (3)	70.5 ± 27.1 (2)	0.392
Trough-1	48.1 ± 7.1 (3)	10.4 ± 7.4 (3)	**0.003**
Trough-2	89.6 ± 10.4 (3)	75.9 ± 13.3 (3)	0.236

Table 3.7. Comparison of final average size at day 56 (mean ± SD mm) for CARC and NANFH treatments averaged from replicates (n) per treatment using Welch's two sample t-test for unequal variance.

	CARC	NANFH	Pr(<t)
Dogpan	8.69 ± 0.12 (3)	5.83 ± 0.14 (3)	**<0.001**
Airlift Upweller	10.38 ± 0.54 (3)	7.06 ± 0.04 (2)	**0.007**
Trough-1	6.25 ± 0.34 (3)	4.81 ± 0.09 (3)	**0.013**
Trough-2	9.90 ± 0.70 (3)	6.00 ± 0.05 (3)	**0.010**

Table 3.8. Comparison of average growth rate (mean ± SD mm/day) for CARC and NANFH treatments averaged from all replicates across sampling dates, using Welch's two sample t-test for unequal variance.

	CARC	NANFH	Pr(<t)
Dogpan	0.057 ± 0.028 (3)	0.007 ± 0.018 (3)	**<0.001**
Airlift Upweller	0.076 ± 0.098 (3)	0.027 ± 0.022 (2)	0.139
Trough-1	0.037 ± 0.025 (3)	0.016 ± 0.014 (3)	**0.043**
Trough-2	0.065 ± 0.076 (3)	0.011 ± 0.020 (3)	**0.038**

Table 3.9. Percent survival (mean ± SD) for different size class mussels within troughs at CARC and NANFH averaged from replicates (n) per treatment. Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$). nd = no data

			Day					
Size Class	**Location**	**n**	**0**	**14**	**28**	**44**	**49**	**56**
Small	CARC	3	100	71.2 ± 4.5	57.5 ± 6.7	n.d	49 ± 5.2	48.1 ± 7.1[b]
Large	CARC	3	100	93.0 ± 2.4	90.3 ± 7.0	n.d	89.3 ± 8.0	89.6 ± 10.4[a]
Group Pr(>F)								<0.001
Day								
Size Class	**Location**	**n**	**0**	**14**	**28**	**44**	**49**	**56**
Small	NANFH	3	100	70.5 ± 11.3	43.8 ± 21.4	21.1 ± 9.7	n.d	10.4 ± 7.4[b]
Large	NANFH	3	100	96.3 ± 1.7	91.6 ± 2.9	87.9 ± 5.4	n.d	75.9 ± 13.3[a]
Group Pr(>F)								<0.001

Table 3.10. Mussel growth rate (mean ± SD mm/day) for different size class mussels within troughs at CARC and NANFH averaged from replicates (n) per treatment. Bold indicates significant group difference using one-way ANOVA ($p<0.05$). Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$). nd = no data.

			Sampling Interval (days)						
Size Class	**Location**	**n**	**0-14**	**14-28**	**28-44**	**28-49**	**44-56**	**49-56**	**Average Growth Rate**
Small	CARC	3	0.023 ± 0.004	0.070 ± 0.001	nd	0.043 ± 0.008b	nd	0.012 ± 0.023	0.037 ± 0.025b
Large	CARC	3	-0.009 ± 0.012	0.163 ± 0.015	nd	0.093 ± 0.012a	nd	0.013 ± 0.055	0.065 ± 0.076b
Group Pr(>F)									<0.001
Small	NANFH	3	0.016 ± 0.011	0.023 ± 0.007	-0.001 ± 0.006b	nd	0.024 ± 0.017b	nd	0.016 ± 0.014b
Large	NANFH	3	0.029 ± 0.01	0.002 ± 0.019	-0.010 ± 0.011b	nd	0.021 ± 0.009b	nd	0.011 ± 0.020b
Group Pr(>F)									<0.001

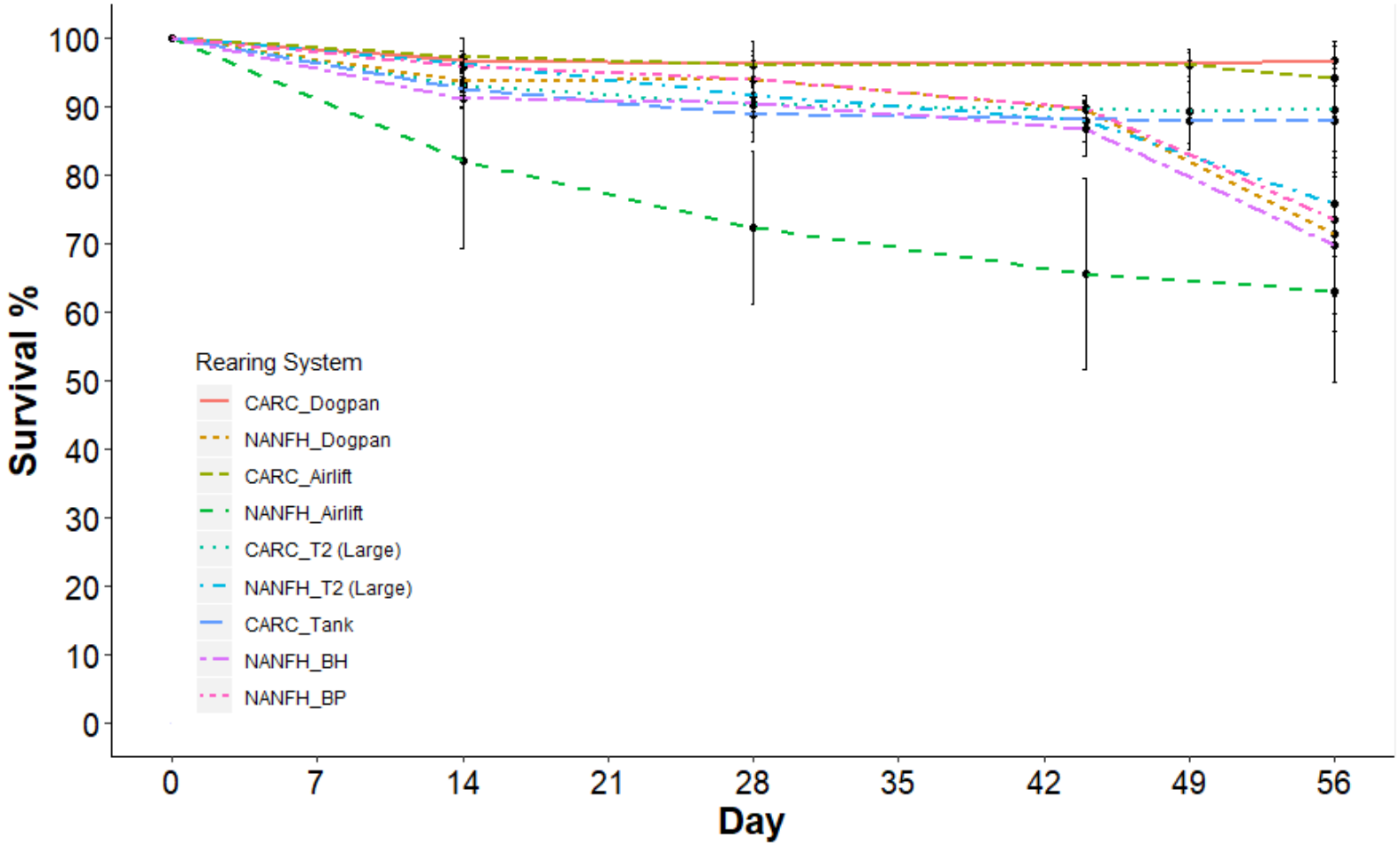

Figure 3.1. Average survival (mean ±SE) for rearing systems on three initial replicates per treatment (except CARC tank upweller that had four replicates and NANFH airlift upweller that had two replicates) per sampling date.

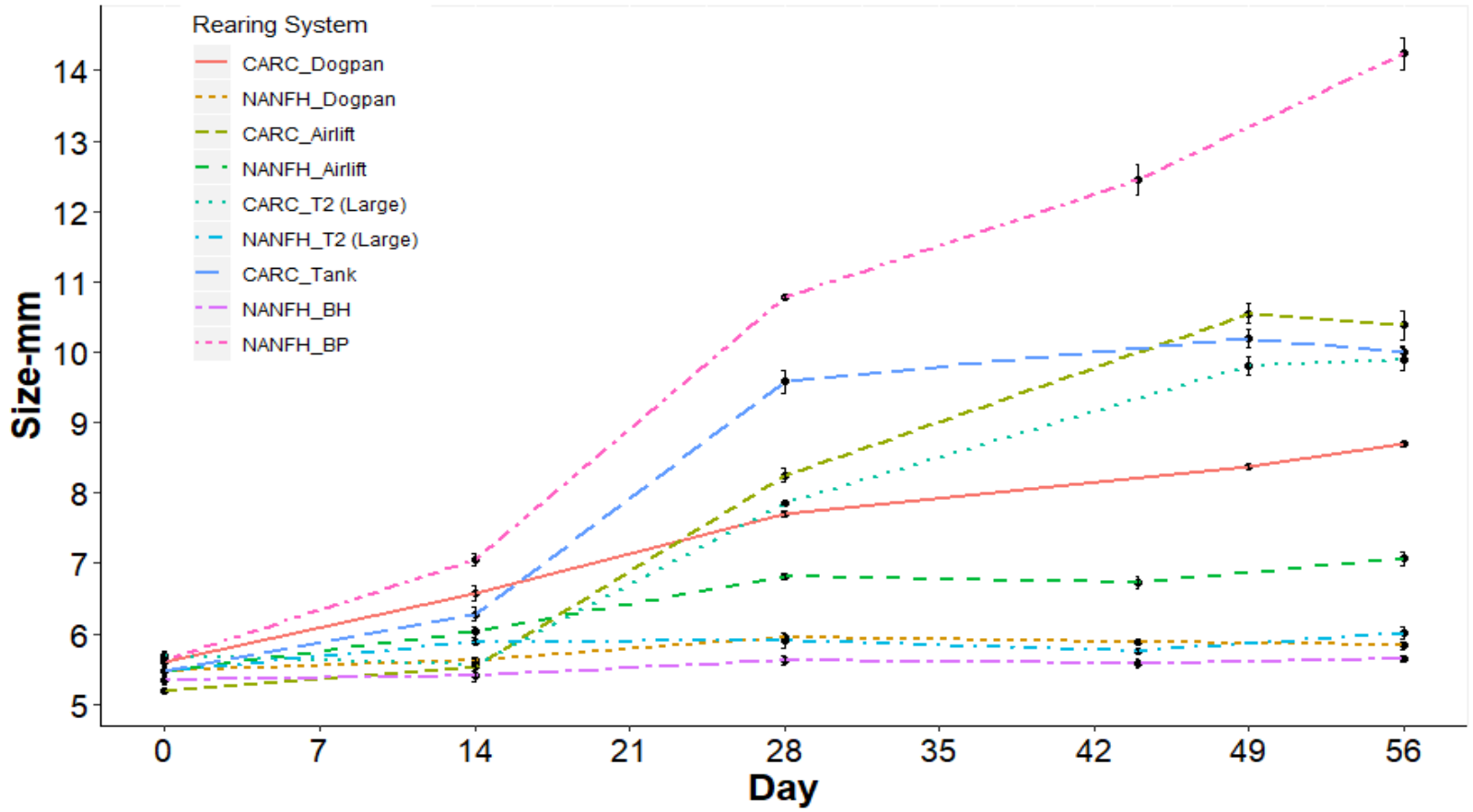

Figure 3.2. Average size (mean ±SE) for rearing systems on three initial replicates per treatment (except CARC tank upweller that had four replicates and NANFH airlift upweller that had two replicates) per sampling date.

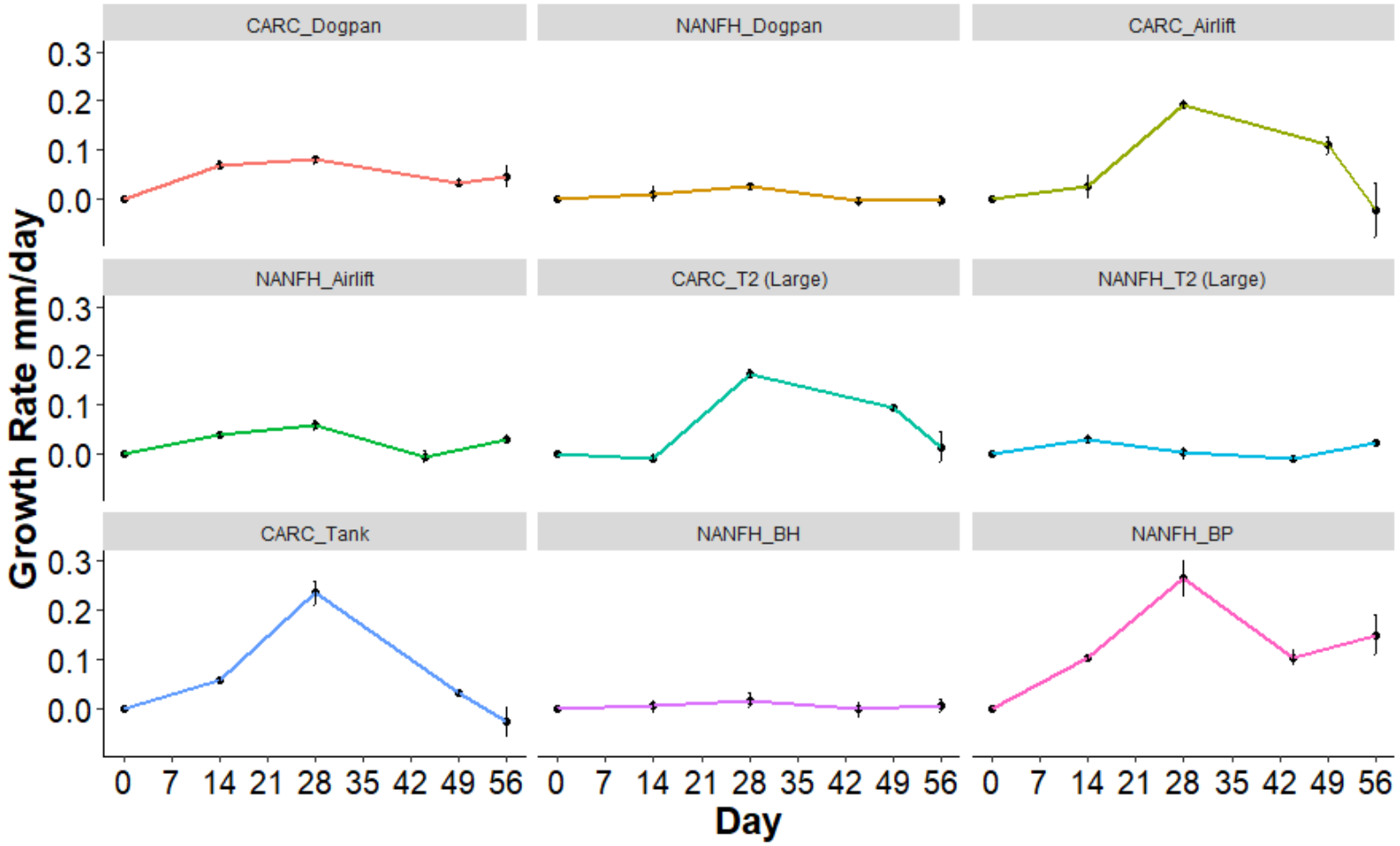

Figure 3.3. Average growth rate (mean ±SE) for rearing systems on three initial replicates per treatment (except CARC tank upweller that had four replicates and NANFH airlift upweller that had two replicates) per sampling date.

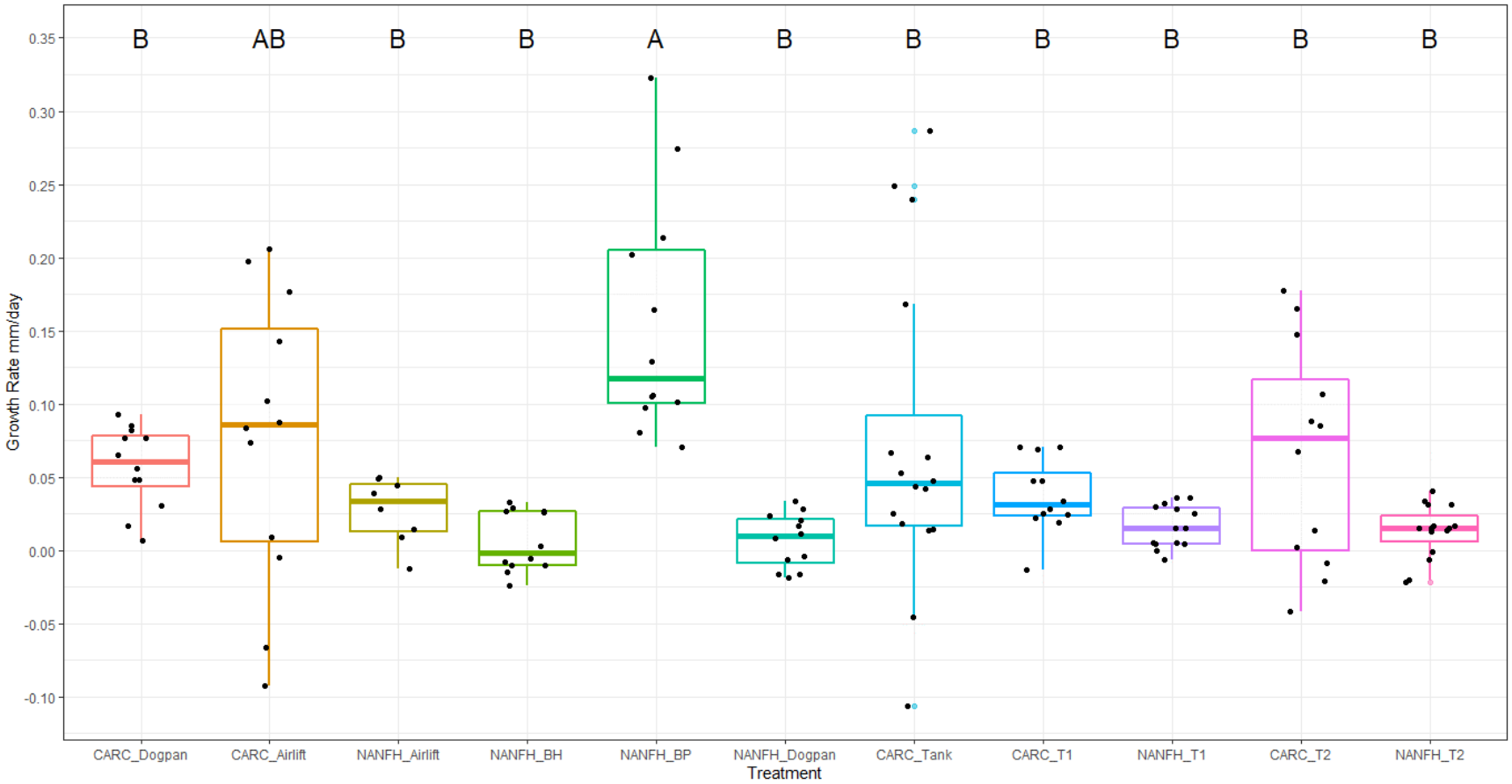

Figure 3.4. Range of juvenile mussel growth rates for rearing system data. Black dots represent calculated growth rate for each replicate per sampling interval.

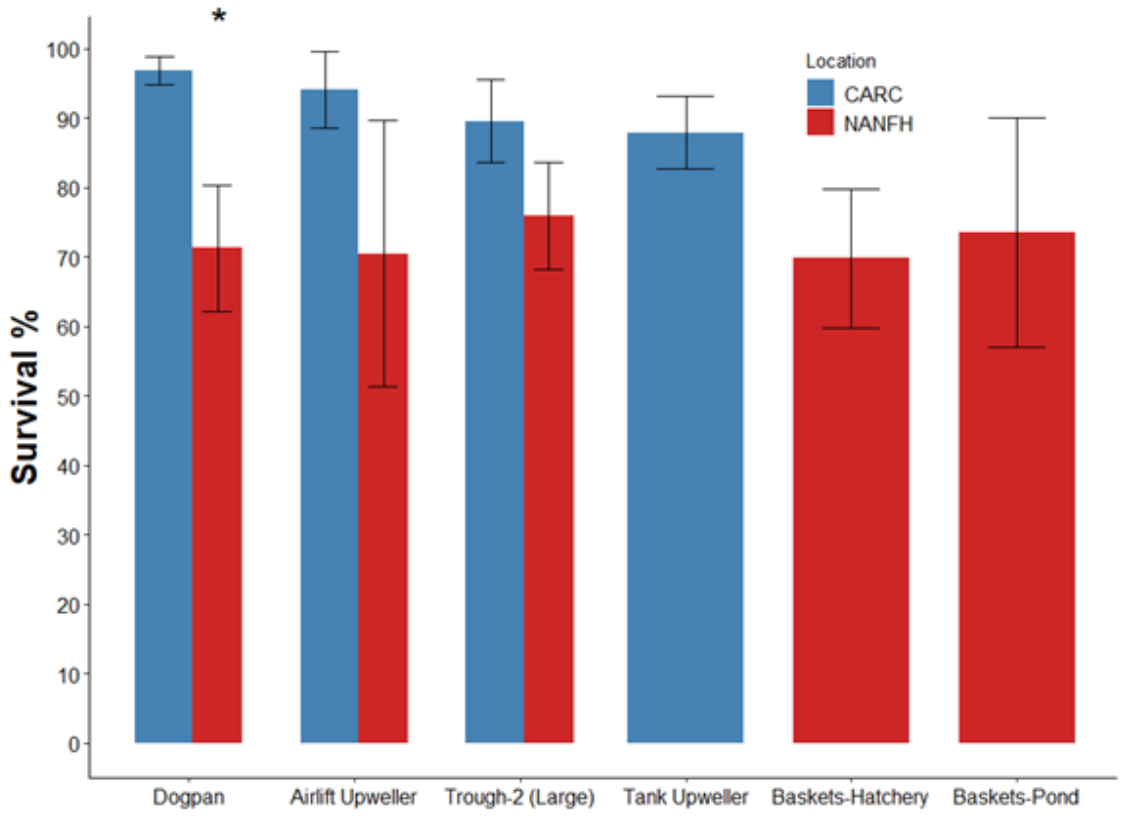

Figure 3.5. Comparison of average survival (mean ±SE) after 56 days for CARC and NANFH rearing systems based on three initial replicates per treatment except NANFH airlift upweller (n=2), tank upweller (n=4), and pond baskets (n=2). P-values (*<0.05) are derived from Welch's two sample t-test for unequal variance.

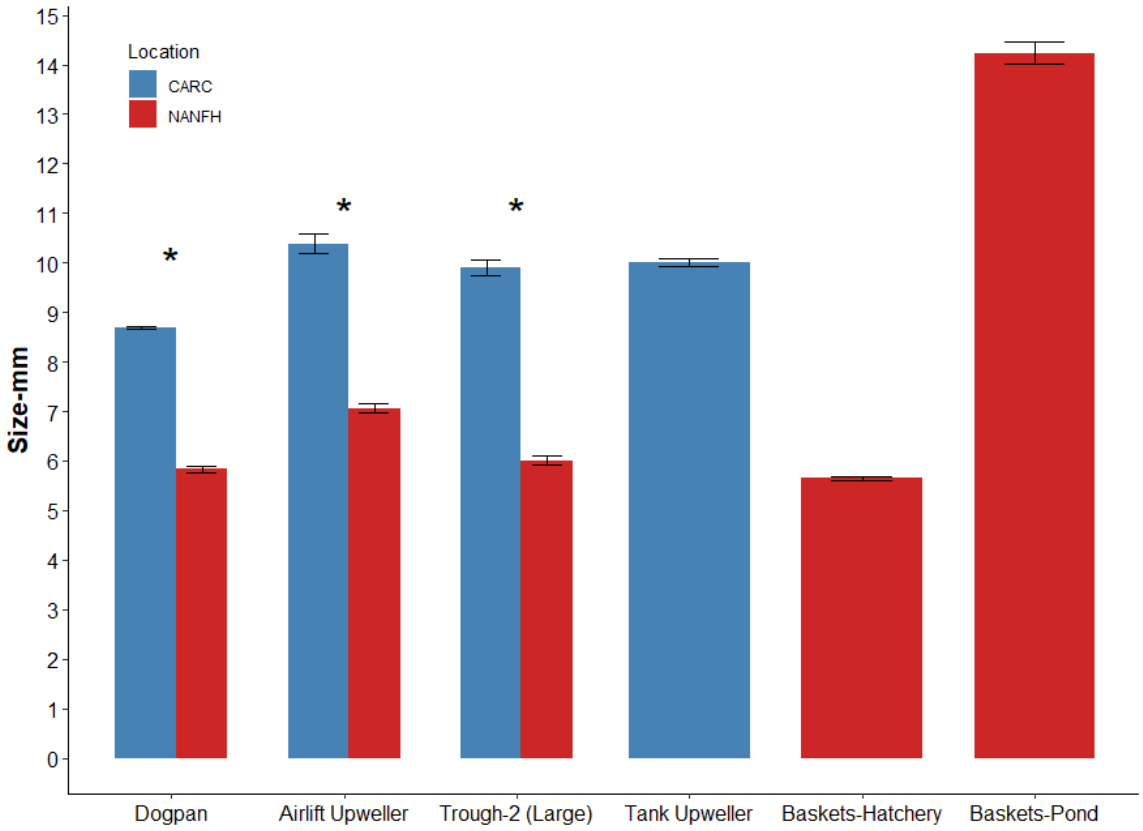

Figure 3.6 Comparison of size (mean ±SE) after 56 days for CARC and NANFH treatments based on three initial replicates per treatment except NANFH airlift upweller (n=2), tank upweller (n=4), and pond baskets (n=2). P-values (*<0.05) are derived from Welch's two sample t-test for unequal variance.

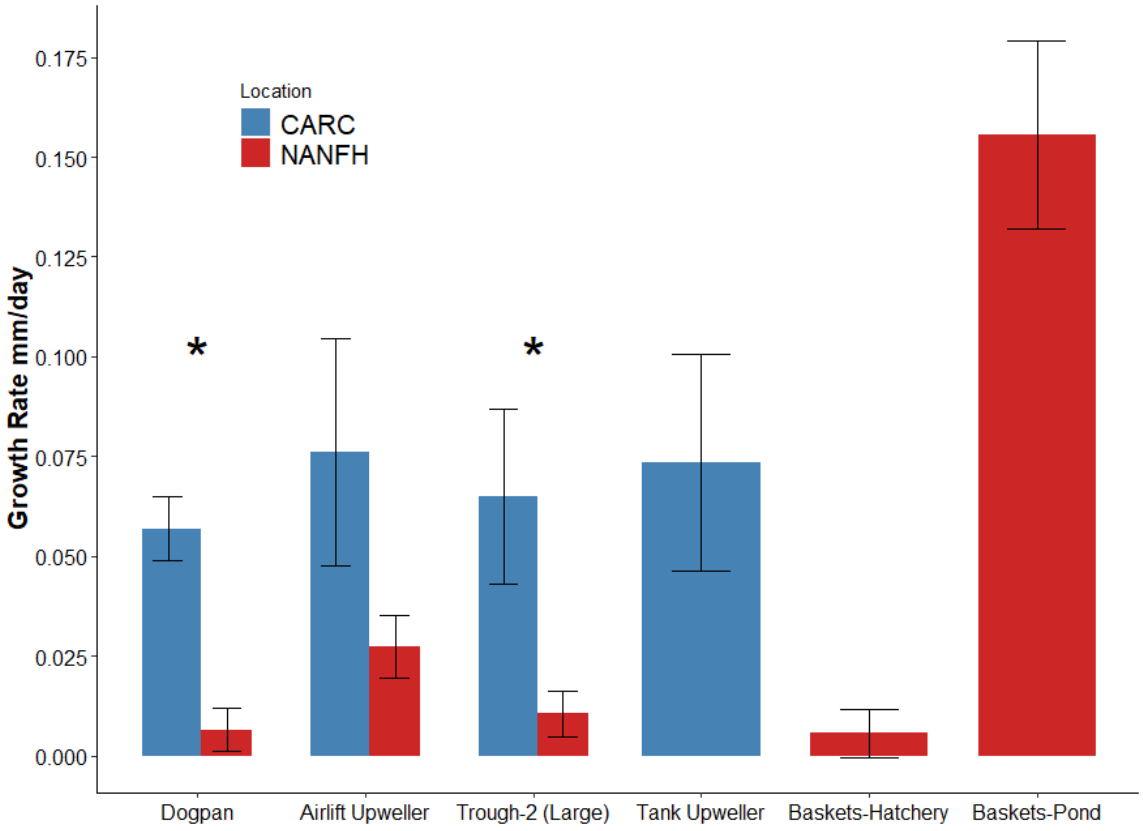

Figure 3.7. Comparison of average growth rate (mean ±SE) for CARC and NANFH treatments based on three initial replicates per treatment except NANFH airlift upweller (n=2), tank upweller (n=4), and pond baskets (n=2). P-values (*<0.05) are derived from Welch's two sample t-test for unequal variance.

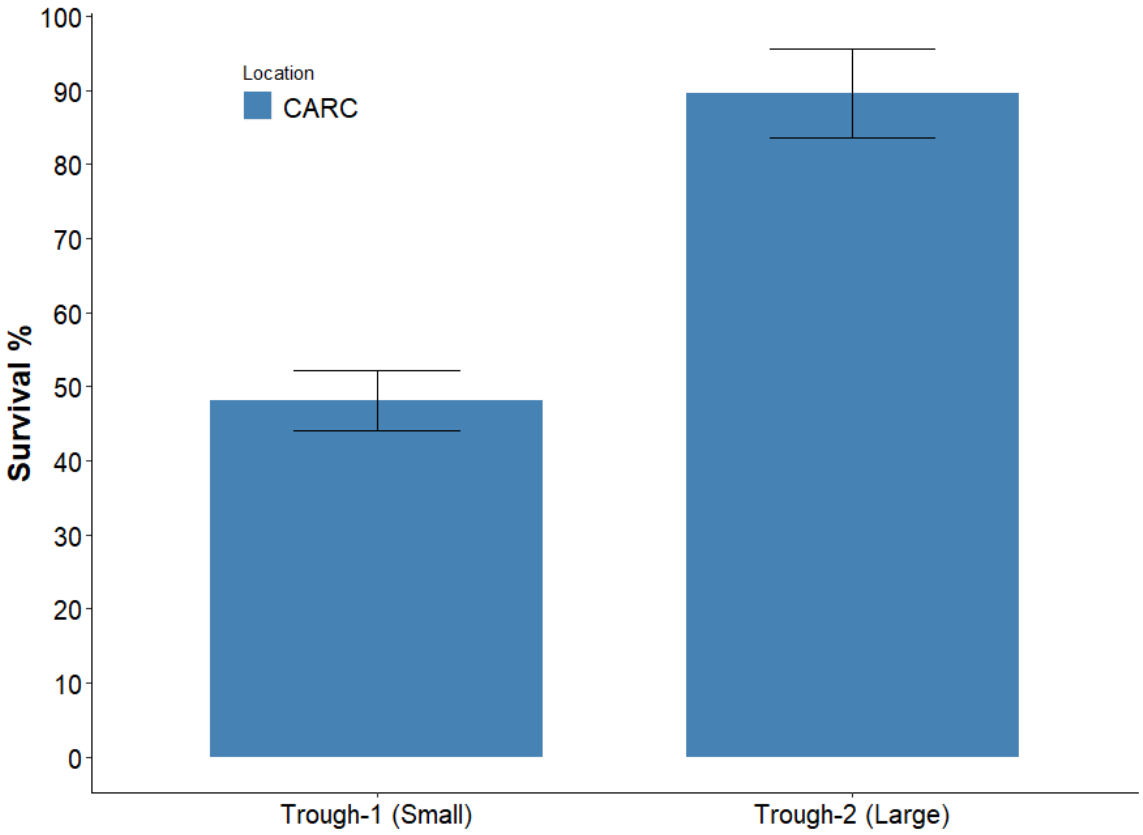

Figure 3.8. Comparison of average survival (mean ±SE) for CARC small and large class size treatments based on three initial replicates per treatment.

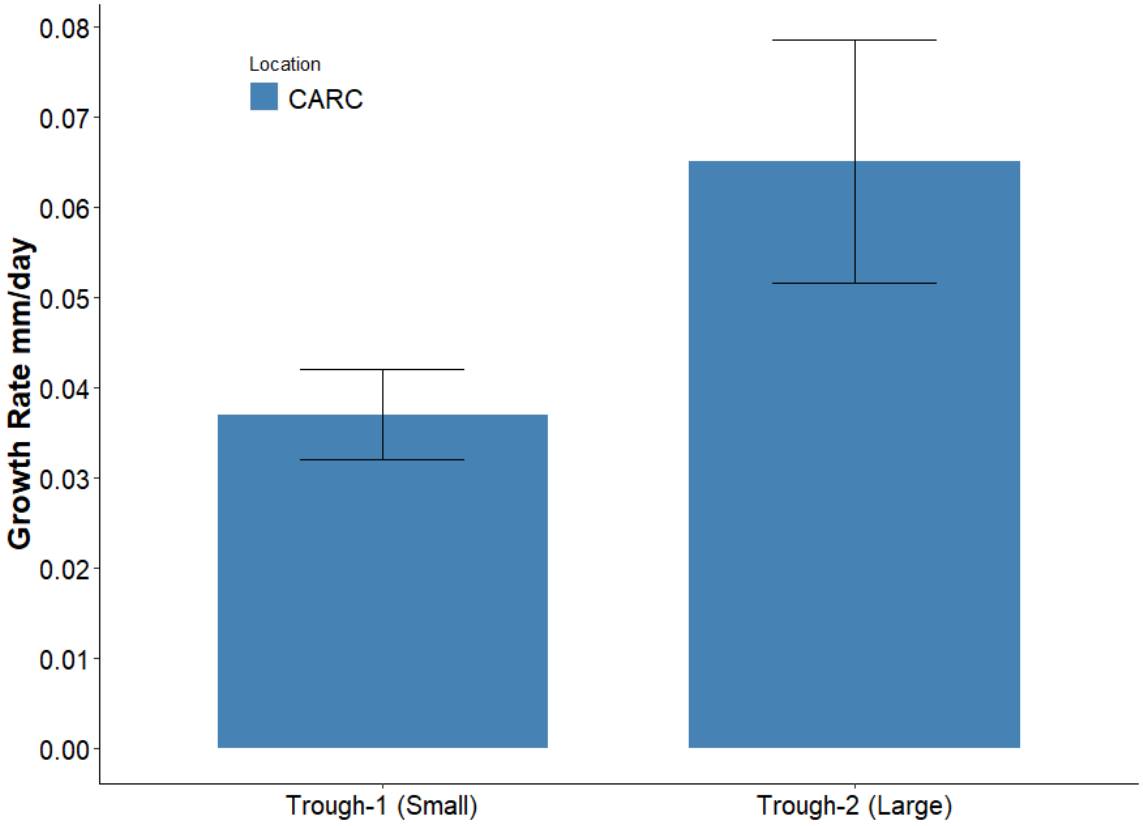

Figure 3.9. Comparison of average growth rate (mean ±SE) for CARC small and large class size treatments based on three initial replicates per treatment.

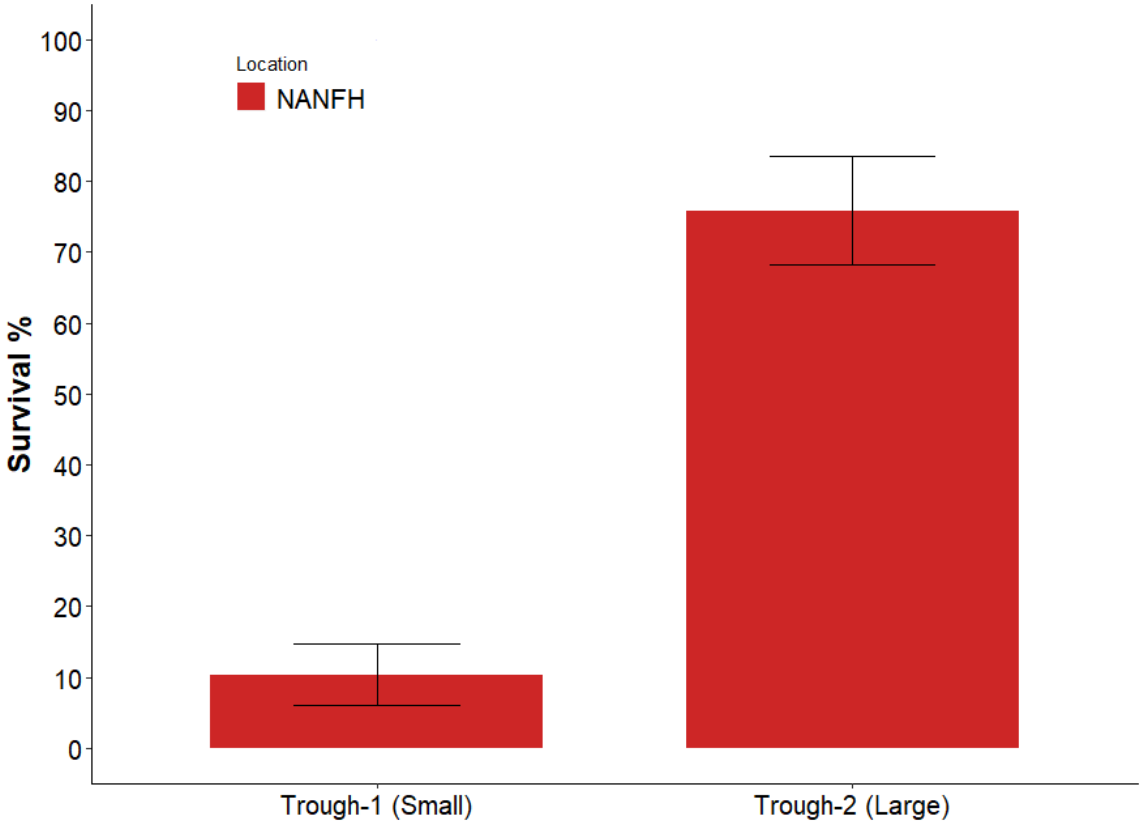

Figure 3.10. Comparison of average survival (mean ±SE) for NANFH small and large class size treatments based on three initial replicates per treatment.

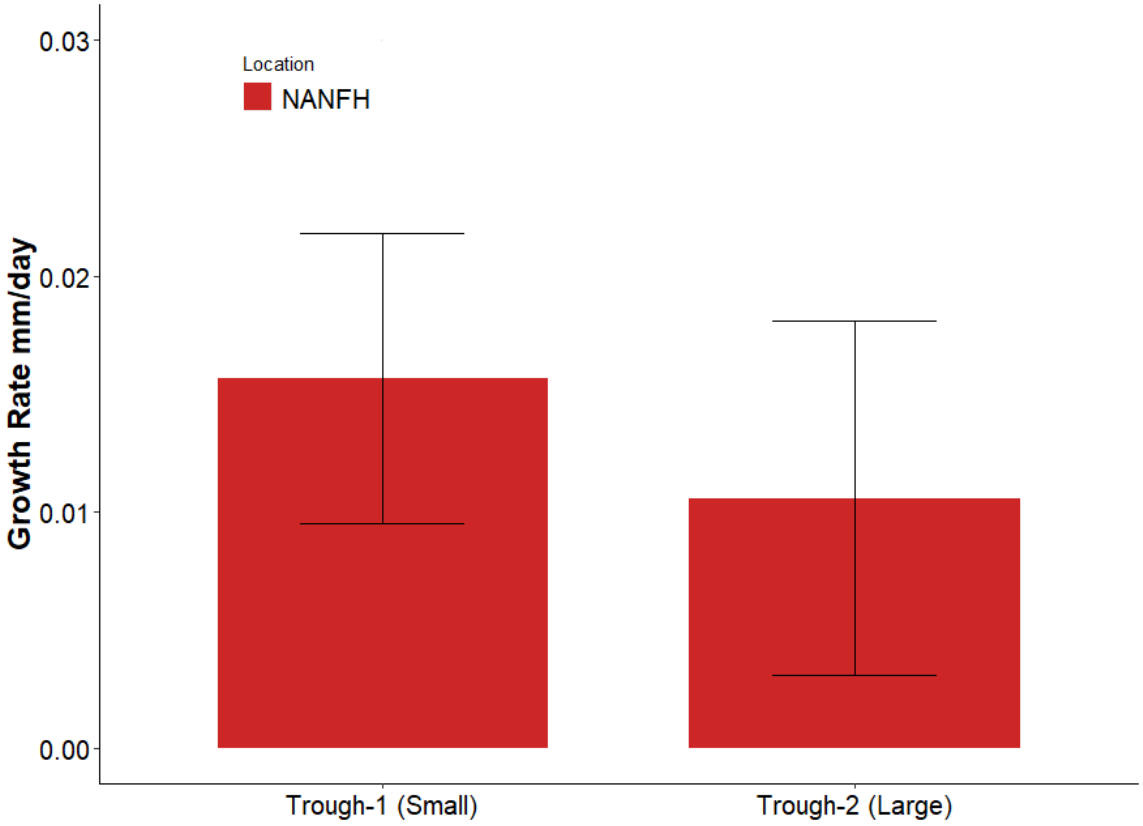

Figure 3.11. Comparison of average growth rate (mean ±SE) for NANFH small and large class size treatments based on three initial replicates per treatment.

CHAPTER 4
CONCLUSIONS AND RECOMMENDATIONS FOR THE PROPAGATION AND CULTURING OF YELLOW LAMPMUSSEL

4.1 Synthesis of Major Findings

In Connecticut and Massachusetts, yellow lampmussel populations are restricted to a 80 km stretch Connecticut River from Windsor, Connecticut to Turners Falls, Massachusetts, and are listed as endangered in both states. Propagation and culture of yellow lampmussel may be needed in to augment populations and maintain minimally viable populations. Few studies have been conducted to improve the culture of yellow lampmussel species; therefore, in this thesis I aimed to fill data gaps regarding the use of commercial probiotics and secondary rearing systems in the culture of yellow lampmussel.

Previous studies have tested the use of bacterial supplements in juvenile mussel rearing; however, the results have been inconsistent. Results of this study (Chapter 2) are similar to previous results where growth and survival metrics were inconsistent between experiments. The B1002 mix improved survival and growth in one study, while the B1000 mix improved survival by 19% and the NiteOut mix improved survival by 10% when compared to the Algae Only treatment, although growth rates were not affected by probiotic mix, regardless of concentration. Though I expected probiotics to reduce ammonia (NH_3-N) concentrations and, in turn, improve survival and growth, probiotics did not significantly reduce NH_3-N concentrations. This indicates that probiotics likely improve survival through another mechanism such as improving feed efficiency or through an immunization boost, rather than through water quality improvements.

In the second project (Chapter 3), I tested the effectiveness of secondary rearing system design on survival and growth using a surrogate species, eastern lampmussel. Floating baskets located at the USFWS North Attleboro location had the highest survival and growth rates out of all the rearing systems; however, CARC rearing systems consistently had higher survival and higher growth rates when comparison similar rearing systems across locations. The results of this study and several previous studies, indicate temperature (Beaty 1999, Beaty and Neves 2004, Negishi and Kayaba 2010, Carey et al. 2013) and food (Gatenby et al. 1996, Kovitvadhi et al. 2006) play a significant role in the differences observed among rearing systems.

4.2 Recommendations

I successfully propagated and cultured yellow lampmussel, and several hundred surviving juveniles are now 8-24 months old. During this project, I performed all aspects of mussel culturing and as such, have several recommendations on protocol for the successful propagation and culture of yellow lampmussel. Several of these recommendations are not specific only to yellow lampmussel; and may be used for species beyond the yellow lampmussel and in other culture facilities. These recommendations are based on my experiences along with published literature.

4.2.1 Adult Mussel Collection and Holding

The timing of brood stock collection may impact post-metamorphosis juvenile health; therefore, acquiring gravid adults during the proper season is important. Jones et al (2005) observed that when glochidia of *Epioblasma capsaeformis* were harvested in the fall the glochidia were not fully mature, metamorphosed juveniles were less active,

and survival and growth was significantly lower than juveniles metamorphosed from spring-harvested glochidia.. The ideal timing for several *Lampsilis* species is during the months of March–October in the state of Virginia; however, for northern states mussels may not be found at the surface until April or May (Patterson et al. 2018). During this study collections in June were more successful (i.e., more gravid mussels were found with less effort compared to those done in the fall or winter) than collections in October or December because average water tides were lower, water flow speed was slower, and temperatures warmer than in the fall or winter, providing better conditions for SCUBA divers. While June may be considered nearer the end of the brooding season and fewer gravid females may be available, successful collections may be done in prior months (beginning in March) when temperatures start to warm and females begin to display the fish lure. Therefore, I would recommend juveniles be collected during the spring or early summer, which would be the end of the brood cycle when glochidia are fully mature.

During this study, gravid mussels were kept from weeks to months at CARC in preparation for inoculation procedures. During captivity, the mussels were conditioned to water temperature of 5-7 °C and supplemental food was withheld. The wild water that is used to change the holding water may provide small pulses of food; however, the low food availability decreases feeding efficiency and digestive enzymes. The purpose of this protocol is to minimize metabolism and reduce metabolic waste (when using a static water system) and prevent premature glochidial release; however, the reduced feeding may also reduce the health of the adult gravid mussels and their larvae (McMahon 1991, Helm and Bourne 2004, Patterson et al. 2018). Furthermore, while several genera of mussels (*Cyptogenia, Dromus, Strophitus,* and *Anodonta)* release glochidia with

temperature cues, lampsiline species use fish lures and wait for external triggers to release glochidia. No evidence of early glochidial release has been observed using lampsiline species at CARC.

Substrate provide adult mussels with burrowing substrate, and relaxed conditions reducing energy expenditure associated with gaping and feeding (Patterson et al. 2018); therefore, in if gravid mussels are held for >2 weeks substrate should be provided to maintain the health of gravid females. Additionally, because condition of the adult mussels relocated from natural habitat decreases over time (Gatenby 2000, Newton et al. 2001, Lima et al. 2011); and that nutrient deficiency is observable in as little as 1-2 weeks under food restrictions (Roznere et al. 2014); I recommend using substrate in the holding chamber, supplementing diet with at least 2.0 mg dry weight of algae (Gatenby et al 2013), and regulating water temperature to mimic natural conditions. Sometimes mussels may need to be kept for extended periods of time due to return conditions (such as high water flows), but reducing holding time is ideal. I would suggest minimizing holding time of adult mussels that are actively fed to <2 months and that inoculations procedures take place within 2 weeks of mussel collection.

4.2.3 Host Fish Inoculation and Care

The process of glochidial extraction and host fish exposure is generally standardized across several genera of mussels (Patterson et al. 2018); however, specific details in technique in extraction and exposure may affect glochidia attachment and successful metamorphosis. Glochidia were extracted from gravid adults using the syringe method (Patterson et al. 2018) which is standard for *Lampsilis* genus; however, there were differences in specific details of the extraction procedure. In experiment 2, I

extracted all glochidia from all gravid females at one time, and combined them into a single batch of glochidia, which was then redistributed into several beakers which were then exposed to the host fish (Appendix C). The entire process took upwards of 4-8 h, which means the glochidia were outside of the gravid adult and unexposed to fish for at least 3 h. Because glochidia viability decreases after extraction from the gravid adult (Fritts et al. 2014), I reduced handling time in experiments 2 and 3 by extracting glochidia of one mussel at a time, then exposing those glochidia to one batch of host fish, rather than extracting all mussel glochidia at once. By doing so, the glochidia were only outside of the gravid adult for less than 1 h before being exposed to the host fish.

Fish mucus can cause glochidia to prematurely close before coming into contact with the host fish. In the first experiment, host fish were moved to the inoculation buckets in advance of inoculation and then glochidia were added to the buckets. To reduce the amount of fish mucus in the inoculation bucket, I waited to move fish into the inoculation buckets until after the glochidia were fully extracted. This procedure appeared to help increase initial attachment rate estimates from an average of 25.5% during Experiment 1, to an average of 72.5% during Experiment 3 (Martell 2018, 2019).

Studies of yellow lampmussel have provided inconsistent data concerning the ideal host fish species; however, candidates for propagation have been identified through laboratory host fish studies, or glochidia identification of host fish in the field, including: white perch (*Morone americana*), yellow perch (*Perca flavescens*) (Wick and Huryn 2002, Wick 2006, Kneeland and Rhymer 2008), largemouth bass (*Micropterus salmoides*) (Eads et al. 2007), black crappie (*Pomoxis nigromaculatus),* white bass *(Morone chrysops),* and striped bass (*Morone saxatilis) (*Eads et al. 2015). During this

study, we used largemouth bass to propagate the yellow lampmussel because they were readily available from fish farms that met disease testing criteria required by the Massachusetts Division of Fisheries and Wildlife. Using certified disease free fish is recommended to ensure a reduction in risk of pathogens and diseases.

Temperature and feeding regiments of the host fish are two factors to consider during the post-inoculation; however, there is no consensus on whether fish require feeding during mussel encystment. Feeding is often withheld to reduce fecal matter in the containment system and weaken the immune response of the host fish (Dodds and Whiles 2004). There is also evidence that host fish condition (Österling and Larsen 2013) and duration of parasitic phase (Marwaha et al. 2016) can affect glochidia metamorphosis (Österling and Larsen 2013); therefore, reducing stress through feeding may produce more robust juveniles. At CARC, fish were not fed during Experiment 1, and for Experiments 2 and 3 fish were fed prior to inoculation and for one week after inoculation. Similar feeding procedures to experiment 2 and 3 have been used in Jacobson et al. (1993), Hanlon (2000), Mummert (2001), and Taeubert et al. (2012). Experiment 1 experienced high mortality due to extended period of time without food; and juvenile mussels were likely lost due to this. By stopping feeding 1 week after inoculation in experiment 2 and 3, the fecal matter was reduced before juvenile drop-off (necessary for ease of counting), while also having the benefit of a reduced fish mortality and mussel loss relative to unfed fish.

4.2.4 Juvenile Rearing

Juvenile rearing significantly impacts the success of juvenile mussel culture. Primary rearing of juvenile mussels is used for newly metamorphosized juveniles and

may be used to mitigate mortality from problems such as mussel wash-out or escapement, ammonia build-up, and predation Prior to experiments, all juveniles were housed in similar 6-inch PVC chambers as part of a recirculating system made of a 22.0 L algae feed bucket, a peristaltic pump for food delivery, and discharge lines. Mussels were fed a standard mussel diet of Marine Microalgae Concentrates 1.5-mL Shellfish Diet 1800 and 0.75-mL Nanno 3600 to 20 L of wild water. After initial drop-offs density of juveniles was approximately 5,000 mussel (of average size 250-280 µm) per chamber (area 1.18 $x10^7$ mm^2) based on holding densities at other mussel facilities (Warren, USFWS, pers.comm). By week 3, differences in individual mussel size was noticeable; however, due to high mortality (>50%) mussel densities decreased over time. Only when mussels grew large enough (>500 µm) were mussels separated into less-dense clusters of about 500 mussels per chamber (generally, 8-12 weeks after initial drop-off). While it is unclear whether density influenced mortality, reducing the density of mussels may be advisable.

There was no clear indication of which probiotic would be the most beneficial in freshwater mussel culture. In the first experiment, the B1002 probiotic improved growth and survival over the algae only treatments; however, in experiment 2 and 3, treatments using B1002 juveniles had lower survival and growth compared to the algae only treatments. In experiment 2, a similar probiotic mix (B1000) improved survival and growth rates of juveniles relative to treatments with only algae. The NiteOut probiotic improved survival and growth rates over an algae only treatment in Experiment 2, and in Experiment 3 the NiteOut probiotic had similar survival results to the algae only treatments, yet had significantly lower growth rates than the algae only treatments. Based on the evidence of this study, to maintain survival of cultured juvenile mussels, I suggest

adding 0.5 ml of NiteOut probiotic per 20 L of algae feed on the first day of juvenile drop-offs. Adding probiotics to the first day of juvenile drop-off may increase immune response to potential pathogens, increase feeding efficiency (WenYing et al. 2009, Bianchi et al. 2017) or provide nutritional benefits (Nichols and Garling 2000) . Further studies in the use of probiotics are recommended, specifically, whether the timing of probiotic administration (e.g. mussel age) effects survival or growth.

Multiple marine mussel studies (synthesized by Prado et al. 2010, Kesacodi-Watson et al. 2008, Sicuro 2015) and 1 freshwater mussel study (Gill 2016 unpublished) have used bacterial analysis of the gastrointestinal tract to identify potential probiotic strains by testing in pathogenic assays, or directly administering as a probiotic to determine mussel growth and survival. Though culturing bacteria from the fut content of adult mussels is more intensive than using commercially-available bacteria, it may provide significant benefits over the use of general probiotics, and further research may be needed to identify population species specific probiotics.

Based on the results of the secondary rearing study (Chapter 3), I would suggest that mussels reared in Massachusetts be moved to the ponds at NANFH, after reaching a size of >5.0 mm, in early April or May to maximize growth. Larger mussels have a significantly better chance at surviving over winter in outdoor rearing systems if they put on enough mussel “weight” prior to winter (Hanlon 2000); however, if mussels are kept at CARC, the dogpan system provided adequate growth and survival up to about 11 mm in size. During the last 3 years I have observed that growth rates of eastern lampmussel kept in dogpans slowed and possibly ceased; therefore, further research into long-term rearing at CARC is needed for when mussels outgrow the current systems. Additionally,

further research into food availability, organic content, and general water quality of the pond may be useful in determining whether culture conditions can be mimicked at other rearing facilities.

4.3 Conclusion

Before beginning a propagation program for species restoration, several variables must be taken into account to determine if propagation and culture of freshwater mussels for population augmentation or reintroduction is justified (McMurray and Roe 2017, Strayer et al. 2019). Understanding the habitat requirements, habitat availability, and host-fish dynamics, and host-fish availability near potential mussels populations or reintroduction sites should be primary research points before attempting a mussel propagation plan. Identification of habitat requirements and suitable habitat locations will aid in population recovery by ensuring conditions for survival, growth, reproduction, and recruitment are met; however; this aspect should be coupled with understanding causes of historical decline including threats to current and future populations. Although determining causes of historical declines is often difficult, some understanding is needed to determine if identifiable threats have been mitigated to ensure future populations are not vulnerable to the same threats. Furthermore, undertaking propagation efforts for population restoration should only be done if there is a plan for long-term protection or habitat restoration for the species of concern (McMurray and Roe 2017).

To effectively enact a conservation plan for a specific species, genetic patterns and diversity of populations should be studied. Because information on dispersal of freshwater mussels is limited, identifying genetic information is the best route in defining population boundaries (Kelly and Rhymer 1005). Homogenization of populations,

decreasing genetic variability within populations, and decreasing fitness of populations through outbreeding depression are all risks associated with population augmentation through captive culture (Jones et al. 2006). In order to maintain genetic diversity and understand the risks associated with population augmentation through propagation (FMCS 2016, McMurray and Roe 2017), the genetic structure of yellow lampmussel of the Connecticut River watershed should be studied. Kelly and Rhymer (2005) found that the genetic structure of yellow lampmussel populations in three river drainages of Maine were significantly different, and populations within 36 km in the same river drainage were different from each other; therefore, different drainages and groups separated by significant distance may need to be treated as individual populations to maintain population level genetics (Kelly and Rhymer 2005). Because isolation by distance has produced genetically distinct populations, it would be imperative to sample within the Connecticut River watershed to determine if genetically distinct populations exist. While there is little evidence in Maine indicating dams influenced population genetics (Kelly and Rhymer 2005), it is plausible that the dams influence genetic dispersal due to host fish migration patterns (Watters 199). Furthermore, to maintain genetic diversity of populations multiple gravid females should be used to inoculate host fish as the release of juveniles from a single female (or few females) may represent a small portion of the genetic diversity within the population (Ryman and Laikre 1991).

Freshwater mussel programs are needed to mitigate the loss of endangered species and maintain species diversity. Propagation of freshwater mussels for population restoration and augmentation is a necessary strategy to preserve the multitude of ecosystem service provided by freshwater mussels. This study aimed to provide methods

to improve culture methods for yellow lampmussel; and similar species in general, aiding to achieve some of the overarching goals outlined by the Freshwater Mollusk Conservation Society in the National Strategy for the Conservation of Native Freshwater Mollusks (FMCS 2016).

APPENDICES

APPENDIX A
PRODUCT DESCRIPTION OF PROBIOTICS USED

Table A.1. Product description of each probiotic used in Chapter 2 Experiments 1,2, and 3. ACF= Alken Clear-Flo, CFU= colony forming units, n/a=not applicable, n.d.=no data available. * indicates probiotic Ammonia Oxidation Rate: 500 mg NH3/liter/hour, and ** indicates probiotic Biological Oxygen Demand: <200 ppm

Probiotic Name	**ACF 1000**	**ACF 1002**	**ACF 1008**	**ACF N1100-50X***	**Glosso Factory**	**NiteOut**
Number of Bacterial Strains	4 gram positive	11 gram positive	11 gram positive, 5 gram negative	2 gram negative	90-95	n.d.
Major Bacterial Groups	*Bacillus*	*Bacillus*	*Bacillus*	*Nitrosomonas europaea* and *Nitrobacter winogradskyl*	*Nitrosomonas* and *Nitrobacter*	*Nitrosomonas*, *Nitrospira*, and *Nitrobacter*
Bacterial Count (CFU/gram)	$2x10^9$	$2x10^{10}$	$4x10^9$	n/a	n.d.	n.d.
Number of fungi strains	n/a	n/a	2	n/a	n/a	n/a
Fungi Count (CFU/gram)	n/a	n/a	$5x10^4$	n/a	n/a	n/a
Bacteria Shape	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped, pear-shaped, or pleomorphic	*Nitrospira* helical to vibroid, *Nitrosomonas* and *Nitrobacter* rod-shaped, pear-shaped, or pleomorphic
Bacteria Size (L x W µm)	1.0-4.0 x 0.5-1.0	1.0-4.0 x 0.5-1.0	1.0-4.0 x 0.5-1.1	0.5-4.0 x 0.3-2.0	0.5-4.0 x 0.3-2.0	0.5-4.0 x 0.3-2.0
Form	Powder	Powder	Powder	Liquid	Powder	Liquid
Solubility	Moderate	Very	Moderate	Completely	Moderate	Completely
Appearance	Tan or brown	Tan or brown	Tan or brown	Turbid and tan	Tan or brown	Light pink
Temperature requirement °C	7-60	5-35	7-37	9.5-65	9.5-65	9.5-65
pH requirement	5.9-9.0	4.5-8.5	5.5-8.5	6.5-8.5	6.5-8.5	6.5-8.5
Dissolved Oxygen tolerance	Anaerobic	>2.0	>2.0	**	n.d.	n.d.

APPENDIX B
YELLOW LAMPMUSSEL AND EASTERN LAMPMUSSEL BROOD STOCK COLLECTION, CARE, AND RETURN

B.1.0 Yellow Lampmussel (Chapter 2)

B.1.1 Collection and Transportation of Gravid Females

Thirty Yellow lampmussels (*Lampsilis cariosa)* were collected 4 times during 2017 and 2018 (Table 1). During 2017, yellow lampmussel were collected on June 30 and December 5. During 2018, yellow lampmussel were collected on October 1 and December 17 (Table B.1). For the June 30th , December 5th, and December 17th collections an independent biological consulting company, Biodrawversity, LLC, was contracted to aid in identification and retrieval of gravid females. For the October 1st collection Peter Hazelton from the MassWildlife performed identification and collection of gravid females. All mussels were collected from the Connecticut River in Hadley, Massachusetts. For all collections team of 2 to 4 individuals used kayaks and SCUBA diving gear to retrieve female mussels from 1-5 meters below the surface. During the June collection, mussels were found on top of the substrate, displaying their fish lure. During the October and December collections, mussels were collected using tactile searching within the substrate. All collected mussels were brought to the surface where trained technicians assessed gravidity. Gravid mussels were placed in a 5-gal portable cooler filled with fresh river water and portable water bubblers, and non-gravid mussels were returned to the river bottom. Mussels were transported approximately 10 miles (approx. 20 minutes) to the Richard Cronin Aquatic Resource Center (CARC).

Table B.1. Brood stock collection of Yellow Lampmussel. A yellow Hallprint tag was attached to each right valve upon collection. N/A=not applicable (e.g. were not used in inoculations).

Right Tag ID	Length (mm)	Width (mm)	Height (mm)	Date Collected	Inoculation Date	Holding Time Until Inoculation (weeks)	Date Returned	Total Holding Time (weeks)
850	86.74	34.23	60.69	6/30/2017	n/a	n/a	9/18/2017	11.5
851	86.87	40.03	60.15	6/30/2017	n/a	n/a	9/18/2017	11.5
852	85.38	34.23	60.59	6/30/2017	8/4/2017	5	Deceased	n/a
853	80.98	35.89	60.08	6/30/2017	8/4/2017	5	9/18/2017	11.5
854	76.02	35.26	57.69	6/30/2017	8/4/2017	5	9/18/2017	11.5
855	81.66	35.18	58.16	6/30/2017	n/a	n/a	9/18/2017	11.5
856	79.2	32.91	57.35	6/30/2017	8/4/2017	5	9/18/2017	11.5
857	69.73	32.34	48.04	6/30/2017	n/a	n/a	9/18/2017	11.5
858	78.19	33.17	55.74	6/30/2017	n/a	n/a	12/5/2017	11.5
859	79.54	37.37	56.75	6/30/2017	n/a	n/a	9/18/2017	11.5
860	76.89	30.29	53.2	6/30/2017	n/a	n/a	9/18/2017	11.5
861	63.1	26.5	44.45	6/30/2017	8/4/2017	5	Deceased	n/a
862	71.9	28.5	49.2	6/30/2017	n/a	n/a	9/18/2017	11.5
863	75.2	32.1	55.4	6/30/2017	n/a	n/a	9/18/2017	11.5
864	76.9	34.5	53.5	6/30/2017	8/4/2017	5	9/18/2017	11.5
865	80.3	33.5	59.1	6/30/2017	n/a	n/a	Deceased	n/a
866	79.7	30.6	55.6	6/30/2017	n/a	n/a	9/18/2017	11.5
946	72.67	26.94	52.44	12/5/2017	5/22/2018	24	10/1/2018	43
947	76.41	31.64	58.42	12/5/2017	n/a	n/a	10/1/2018	43
948	69.01	27.81	48.38	12/5/2017	5/22/2018	24	10/1/2018	43
949	73.26	30.25	53.01	12/5/2017	n/a	n/a	10/1/2018	43
950	81.52	38.63	61.54	12/5/2017	5/22/2018	24	10/1/2018	43
951	5.77	37.75	62.68	12/5/2017	5/22/2018	24	10/1/2018	43
954	87.79	39.71	67.01	10/1/2018	2/12/2019	19	6/4/2019	35
955	77.04	36.16	55.73	10/1/2018	n/a	n/a	6/4/2019	35
956	77.51	33.43	55.56	10/1/2018	2/12/2019	19	6/4/2019	35
957	78.91	33.55	58.52	10/1/2018	n/a	n/a	6/4/2019	35
958	75.22	32.84	56.33	10/1/2018	n/a	n/a	6/4/2019	35
967	74.48	30.36	54.49	12/17/2018	2/12/2019	8	6/4/2019	35
968	88.55	39.12	63.83	12/17/2018	2/12/2019	8	6/4/2019	35

B.1.2 Holding Adults

Prior to arrival of the adult mussels at CARC, individual retention containers (1.5L glass mason jars for June 30th brood stock, or 3-9L aquaria for Oct 1st, Dec 5th and Dec 17th brood stock) were filled with water and placed into a refrigerated storage system set to the average weekly temperature of the Connecticut River at the corresponding time of year to prevent adult mussels from experiencing temperature shock. The source water for the adult mussels was a mixture of surface and ground water from the outdoor raceways at CARC that had been pumped into a storage container adjacent to the hatchery building.

Upon arrival of mussels at CARC, water temperature of the portable cooler water was compared with the retention containers to insure similarity. Adult mussels were then transferred into retention containers, which were each equipped with air stones to oxygenate the water. The water temperature of retention containers was decreased at a rate of 2°C per day until the holding water was approximately 5°C, which simulated fall and winter temperature when adult mussels retain their glochidia, and hence prevented release of glochidia before our use for propagation. Holding water was changed once a week to minimize ammonia and waste build-up. New retention containers were filled each week and placed into the refrigerator system so they would be the same temperature for water change-outs the following week. Adult mussels were not fed during holding. For 2018 brood stock, sterilized sediment was added to the retention containers for each mussel.

B.1.3 Documenting Adults

Adult mussels entering the facility were documented and recorded into our database. The height, width, and length of each mussel was taken using digital calipers. Each mussel was inspected for previous identification tags; if untagged, new identification tags were applied. For all brood stock collected yellow Hallprint tags were glued onto the center of the right valve (Figure B.1).

Figure B.1.Yellow Hallprint tag attached to the right valve of yellow lampmussel (*Lampsilis cariosa).*

B.1.4 Returning Adults

All brood stock were returned to the approximate same area as collection. Before the date of return, the temperature in the refrigerator system was adjusted to match the temperature of the water in the Connecticut River. Holding water was adjusted over a period of one to seven days until the corresponding water temperature was achieved. Mussels were transported in coolers equipped with portable water bubblers to the location of collection. All mussels from the June 2017 brood stock were released from the water surface in the approximate location of collection in the Connecticut River in Hadley, Massachusetts.. All mussels from the December 2017 and October/December 2018 mussels were seeded into the substrate in an upright position.

B.2.0 Eastern Lampmussel (Chapter 3)

B.2.1 Collection and Transportation of Gravid Females

Ten eastern lampmussels *(Lampsilis radiata)* were collected from the Mill River in Whately, Massachusetts in August 2016. Collections were done by Timothy Warren (USFWS), Virginia Martell (USFWS), and Stephanie Gill (University of Massachusetts-Amherst). Mussels were collected using mussel view buckets. During the collection, mussels were found on top of the substrate, displaying their fish lure. All mussels were collected between 0.25-1 meter below the surface. All collected mussels were brought to the surface where trained technicians assessed gravidity. Gravid mussels were placed in a 5-gal portable cooler filled with fresh river water and portable water bubblers, and non-gravid mussels were returned to the river bottom. Mussels were transported approximately 7 miles (approx. 10 minutes) to the Richard Cronin Aquatic Resource (CARC).

B. 2.2 Holding Adults, Documenting Adults, and Releasing Adults.

Prior to arrival of the adult mussels at CARC, individual retention containers (1.5L glass mason jars) were filled with water and placed into a refrigerated storage system set to the average weekly temperature of the Mill River at the corresponding time of year to prevent adult mussels from experiencing temperature shock. All other holding conditions were similar to those described for yellow lampmussel. In 2016, eastern lampmussels were not tagged; and were returned to the Mill River in November 2016 by seeding mussels into the substrate in an upright position.

APPENDIX C
YELLOW LAMPMUSSEL AND EASTERN LAMPMUSSEL HOST-FISH INOCULATION, FISH CARE, AND PRIOR JUVENILE REARING

Yellow lampmussel (Chapter 2)

Fish inoculation followed standard published procedures for glochidia harvest, viability assessment, and host-fish inoculation (Patterson et al. 2018). Fish inoculations took place at the Richard Cronin Aquatic Resource Center on August 4, 2017 (Experiment 1); February 12, 2019 (Experiment 2), and May 22, 2018 (Experiment 3). Host-fish inoculation data is summarized in Table C.1.

Fish Collection

Prior to inoculation 400 (experiment 1), 450 (experiment 2), and 210 (experiment 3) largemouth bass were purchased from Hicklings Fish Farm in Edmeston, NY in July 2017 (experiment 1), November 2018 (experiment 2), and May 2018 (experiment 3). At Hicklings, fish were acclimated to the approximate temperature of the transport water to prevent temperature shock. Portable aeration systems were used in the transport tank to maintain dissolved oxygen levels during transportation (~ 4 hours). Upon arrival at CARC, fish were transferred into three to four 288-liter flow-thru circular tanks fed with well water. For experiment 1, fish were not fed after arrival at CARC. For experiments 2 and 3, the fish were fed after arriving at CARC until 1 week after inoculations. We ceased feeding during juvenile drop-offs to reduce fecal matter in the water system during juvenile drop-offs.

Glochidia Estimation and Preparation

For each inoculation, the shell of adult mussels were opened slightly and the gills pierced with a 22-gauge hypodermic needle. Glochidia from each mussel was flushed into separate beakers. Glochidia density and viability was determined through subsampling and a salt test (Patterson et al. 2015). Five, 200-µl samples were taken from each beaker and density was estimated. One drop of salt solution (NaCl) was added to the glochidia in each subsample to estimate viability. Glochidia that close when salt is added to the subsample indicates that they are alive and able to attach to the host fish.

For experiment 1, after all glochidia was flushed from the adult mussels (n=8) and viability was assessed, the glochidia was then combined into a single beaker and redistributed among 16 beakers before being applied to the fish inoculation buckets (batch 1 and batch 2). For experiments 2 and experiments 3, glochidia was flushed from a single mussel prior to each batch, rather than flushing all mussels at once. This was to reduce the time glochidia was spent outside the mussel before being applied to the host-fish.

Inoculation Procedures

Experiment 1

Twenty-five largemouth bass were placed into 19-liter buckets supplied with well water and equipped with an air stone. Glochidia was then added to the inoculation buckets. Fish were exposed to glochidia for 25 minutes while the water was continually mixed using turkey basters to keep glochidia in suspension. Following inoculation, bass

were divided equally into four 288-liter circular tanks kept at an average temperature of 19°C.

Experiment 2 and 3

Instead of keeping host-fish in the inoculation buckets prior to readiness of the glochidia, largemouth bass were pre-sorted and moved from primary holding tanks to secondary holding tanks for quicker transfer. Once the glochidia was fully extracted and prepared, 13-27 largemouth bass were placed in each 19-liter bucket supplied with well water and equipped with an air stone. Glochidia was then added to the inoculation buckets. Fish were exposed to glochidia for 30 minutes while the water was continually mixed using turkey basters to keep glochidia in suspension. Following inoculation, bass were divided equally into four 288-liter circular tanks kept at an average temperature of 22°C for Experiment 2; and 19°C for Experiment 3.

Table C.1. Mussel brood stock ID, collection date, inoculation date, and holding time for production of juvenile yellow lampmussel (*Lampsilis cariosa)*.

Brood stock ID	Collection Date	Inoculation Date	Holding Time Until Inoculation (weeks)
Inoculation 1			
852	6/30/17	8/4/17	5
853	6/30/17	8/4/17	5
854	6/30/17	8/4/17	5
856	6/30/17	8/4/17	5
861	6/30/17	8/4/17	5
864	6/30/17	8/4/17	5
Inoculation 2			
946	12/5/17	5/22/18	24
948	12/5/17	5/22/18	24
950	12/5/17	5/22/18	24
951	12/5/17	5/22/18	24
Inoculation 3			
954	10/1/18	2/12/19	19
956	10/1/18	2/12/19	19
967	12/17/18	2/12/19	8
968	12/17/18	2/12/19	8

Table C.2. Summary of host-fish inoculation data for juveniles produced for experiment 1 (Inoculation 1), experiment 2 (Inoculation 2), and experiment 3 (Inoculation 3) (Chapter 2).

	Gravid mussels (n)	Host Species	# Inoculation buckets	# Fish per bucket	Water volume (L)	Viable glochidia per liter	Viable glochidia per fish	Exposure Time
Inoculation 1								
Batch 1	8	Largemouth bass	8	25	10	5291	2117	25
Batch 2		Largemouth bass	8	25	3	22421	2690	25
Inoculation 2								
Batch 1	1	Largemouth bass	6	15	1.5	10000	1000	30
Batch 2	1	Largemouth bass	6	13	1.3	10000	1000	30
Batch 3	1	Largemouth bass	6	25	2.5	10000	1000	30
Batch 4	1	Largemouth bass	6	22	2.2	10000	1000	30
Inoculation 3								
Batch 1	1	Largemouth bass	2	27	3	20766	2192	30
Batch 2	1	Largemouth bass	3	26	3	17250	1951	30
Batch 3	2	Largemouth bass	3	26	3	33337	3750	30

Fish Care and Euthanasia

Fish tanks were checked daily to record temperature, identify fish diseases, and remove any deceased fish. Salinity was recorded weekly. After final juvenile collections were done (determined by visual inspections of five to ten fish from each tank to evaluate juvenile attachment, and a decrease in individual juveniles collected over previous days), fish were euthanized using MS-222 following the Institutional Animal Care and Use Committee (IACUC) protocol #2016-0075 at the University of Massachusetts Amherst.

Eastern lampmussel (Chapter 3)

Fish inoculation followed standard published procedures for glochidia harvest, viability assessment, and host-fish inoculation (Patterson et al. 2018). Fish inoculations took place at the Richard Cronin Aquatic Resource Center on October 18, 2016. Host-fish inoculation data is summarized in Table C.3.

Fish Collection

Prior to inoculation 300 largemouth bass were purchased from Hicklings Fish Farm in Edmeston, NY in July of 2016. At Hicklings, fish were acclimated to the approximate temperature of the transport water to prevent temperature shock. Portable aeration systems were used in the transport tank to maintain dissolved oxygen levels during transportation (~ 4 hours). Upon arrival at CARC, fish were transferred into three to four 288-liter flow-thru circular tanks fed with well water. Fish were not fed after arrival at CARC.

Glochidia Estimation and Preparation

For inoculation, the same procedure for glochidia estimate and preparation was used that was previously described for yellow lampmussel. Glochidia was extracted by

opening the mussel shell slightly and piecing the gill with a 22-gauge hypodermic needle. Glochidia density and viability was determined through subsampling and a salt test (Patterson et al. 2015).

All glochidia was flushed from the adult mussels (n=8) and viability was assessed, the glochidia was then combined into a single beaker and redistributed among 16 beakers before being applied to the fish inoculation buckets (batch 1 and batch 2).

Inoculation Procedures

Twenty-five Largemouth bass were placed in each 19-liter bucket supplied with well water and equipped with an air stone. Glochidia was then added to the inoculation buckets. Fish were exposed to glochidia for 25 minutes while the water was continually mixed using turkey basters to keep glochidia in suspension. Following inoculation, bass were divided equally into four 288-liter circular tanks kept at an average temperature of 19°C.

Table C.3. Summary of host-fish inoculation data for juveniles produced for secondary rearing study (Chapter 3).

	Gravid mussels (n)	Host Species	# Inoculation buckets	#Fish per bucket	Water volume (L)	Viable glochidia per liter	Viable glochidia per fish	Exposure Time
Batch 1	8	Largemouth bass	8	25	10	5291	2690	25
Batch 2		Largemouth bass	8	25	3	22421	2117	25

Fish Care and Euthanasia

Fish tanks were checked daily to record temperature, identify fish diseases, and remove any deceased fish. Salinity was recorded weekly. After final juvenile collections were done (determined by visual inspections of five to ten fish from each tank to evaluate juvenile attachment, and a decrease in individual juveniles collected over previous days), fish were euthanized using MS-222 following the Institutional Animal Care and Use Committee (IACUC) protocol #2016-0075 at the University of Massachusetts Amherst.

Juvenile Rearing Prior to Secondary Rearing Study

Before secondary rearing system trials, eastern lampmussels were housed in mini-downwelling chambers. An algae feed mixture was administered at approximately 8.0 mL/min via a Masterflex Peristaltic Pump. The algae feed was a combination of 2.0 mL Marine Microalgae Concentrates, 2.0 mL Shellfish Diet 1800 and 1.0 mL Nanno 3600, mixed with 20 L of wild water. Wild water was acquired from an exterior pond that was a mix of surface water and groundwater. The water was sterilized with a UV light and filtered through 5-µm mesh. Water was completed exchanged every 2 days. An air stone was used to oxygenate the water. The water was kept at approximately 19°C through regulation of ambient air temperature in the lab.

When mussels reached an average size of 4 mm in length (8-10 months in age), they were transferred to a secondary rearing system: a dogpan with recirculating wild water. Water change outs were done once weekly. Water treatment and feed concentration remained the same as described above; however, an additional 1.5 mL of Shellfish Diet and 0.75 mL of Nanno diet was administered three days after water

change-outs. The water flow was approximately 500 mL/min. The temperature was kept approximately 25°C.

APPENDIX D
AMMONIA (NH_3-N MG/L) RATIOS CALCULATED FROM THE AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA FOR AMMONIA (ALAWQCA) USING THE HENDERSON-HASSELBACK EQUATION AT SPECIFIED TEMPERATURE AND PH VALUES

The ALAWQCA (2013) recommended standards represent are given as a concentration of TAN (total ammonia nitrogen) where TAN= NH_4+ NH_3; however, fractional concentrations of NH_4 (ammonium) and NH_3 (ammonia) can be calculated using the Henderson-Hasselback Equation where:

$$\mathrm{NH_4} = \frac{\text{Total ammonia}}{(1+\text{antilog}(\mathrm{pH}-\mathrm{p}K\mathrm{a}))} = \text{Total ammonia} - \mathrm{NH_3}$$

(Wood 1993)

$$\mathrm{p}K\mathrm{a} = 0.09018 + (\frac{2729.92}{273.2 + \text{Temperature}(°\mathrm{C})}$$

(Emerson et al. 1975)

Table D.1. Temperature and pH dependent values of ammonia (NH_3-N mg/L) for the Acute Criterion Magnitude with *Oncorhynchus spp.* absent

	Temperature ℃						
pH	**19**	**20**	**21**	**22**	**23**	**24**	**25**
7.0	0.066	0.067	0.064	0.064	0.064	0.063	0.062
7.1	0.079	0.075	0.075	0.075	0.074	0.073	0.071
7.2	0.087	0.088	0.087	0.087	0.085	0.082	0.081
7.3	0.095	0.094	0.093	0.091	0.093	0.091	0.090
7.4	0.110	0.109	0.104	0.103	0.102	0.101	0.099
7.5	0.115	0.114	0.113	0.112	0.111	0.109	0.108
7.6	0.125	0.123	0.122	0.120	0.119	0.118	0.115
7.7	0.133	0.131	0.130	0.128	0.126	0.124	0.122
7.8	0.139	0.137	0.137	0.135	0.133	0.129	0.128
7.9	0.145	0.144	0.141	0.141	0.140	0.137	0.134
8.0	0.150	0.149	0.148	0.145	0.141	0.141	0.140
8.1	0.155	0.152	0.153	0.148	0.146	0.144	0.140
8.2	0.160	0.160	0.152	0.156	0.152	0.147	0.149

Table D.2. Temperature and pH dependent values of ammonia (NH_3-N mg/L) for the 30-day rolling average Chronic Criterion Magnitude with *Oncorhynchus spp.* absent

	Temperature °C						
pH	**19**	**20**	**21**	**22**	**23**	**24**	**25**
7.0	0.007	0.008	0.008	0.008	0.008	0.008	0.008
7.1	0.009	0.009	0.009	0.009	0.009	0.009	0.009
7.2	0.010	0.011	0.011	0.011	0.011	0.011	0.012
7.3	0.012	0.013	0.013	0.013	0.013	0.014	0.013
7.4	0.015	0.015	0.015	0.015	0.016	0.016	0.016
7.5	0.017	0.017	0.017	0.017	0.018	0.018	0.018
7.6	0.020	0.020	0.020	0.020	0.021	0.020	0.020
7.7	0.022	0.021	0.023	0.023	0.023	0.023	0.023
7.8	0.025	0.024	0.025	0.025	0.025	0.026	0.026
7.9	0.027	0.027	0.028	0.028	0.028	0.028	0.028
8.0	0.030	0.030	0.030	0.030	0.030	0.030	0.031
8.1	0.032	0.032	0.032	0.032	0.032	0.033	0.033
8.2	0.034	0.034	0.034	0.034	0.034	0.034	0.036

Table D.3. Temperature and pH dependent values of ammonia (NH_3-N mg/L) for highest 4-day average Chronic Criterion Magnitude with *Oncorhynchus spp.* absent

	Temperature °C						
pH	**19**	**20**	**21**	**22**	**23**	**24**	**25**
7.0	0.018	0.019	0.019	0.019	0.020	0.020	0.020
7.1	0.022	0.022	0.023	0.023	0.023	0.023	0.023
7.2	0.026	0.027	0.027	0.027	0.027	0.027	0.029
7.3	0.031	0.031	0.032	0.032	0.032	0.034	0.034
7.4	0.037	0.037	0.037	0.037	0.040	0.039	0.039
7.5	0.043	0.043	0.043	0.043	0.046	0.045	0.044
7.6	0.051	0.051	0.050	0.049	0.053	0.051	0.051
7.7	0.054	0.054	0.058	0.056	0.057	0.057	0.058
7.8	0.063	0.061	0.062	0.063	0.063	0.064	0.064
7.9	0.068	0.068	0.069	0.070	0.070	0.070	0.070
8.0	0.074	0.075	0.075	0.075	0.075	0.076	0.077
8.1	0.079	0.080	0.080	0.081	0.080	0.081	0.084
8.2	0.084	0.084	0.086	0.085	0.085	0.085	0.089

APPENDIX E
MODEL EVALUATION FOR LOGISTIC REGRESSON OF SURVIVAL DATA

Table E.1. Model evaluation for logistic regression of survival data for experiment 1(Chapter 2).

	Log-Likelihood	χ^2	df	Pr>χ^2	R^2
Likelihood ratio test:	-2677.7	4000.6	10	<0.001	
Wald test: Treatment		658.9	4	<0.001	
Wald test: Day		2111.5	1	<0.001	
Wald test: Treatment*Day		2539.6	4	<0.001	
Goodness-of-Fit					
Hosmer & Lemeshow		2.3189	8	0.9696	
Pseudo-R^2					
McFadden					0.85
Cox & Snell					1.0
Nagelkerke					1.0

Table E.2. Model evaluation for logistic regression of survival data for experiment 2(Chapter 2).

	Log Likelihood	χ^2	df	Pr>χ^2	R^2
Likelihood ratio test:	-4114.1	7061.5	14	<0.001	
Wald test: Treatment		10286	7	<0.001	
Wald test: Day		2869	1	<0.001	
Wald test: Treatment*Day		4956.4	6	<0.001	
Goodness-of-Fit					
Hosmer & Lemeshow		441	18	0.5062	
Pseudo-R^2					
McFadden					0.89
Cox & Snell					1.0
Nagelkerke					1.0

Table E.3. Model evaluation for logistic regression of survival data for experiment 3 (Chapter 2).

	Log Likelihood	χ^2	df	Pr>χ^2	R^2
Likelihood ratio test:	-1785.4	2247.7	16	<0.001	
Wald test: Treatment		5502.1	8	<0.001	
Wald test: Day		1106.7	1	<0.001	
Wald test: Treatment*Day		1107.2	7	<0.001	
Goodness-of-Fit					
Hosmer & Lemeshow		7.77	8	0.4561	
Pseudo-R^2					
McFadden					0.86
Cox & Snell					1.0
Nagelkerke					1.0

Table E.4. Model evaluation for logistic regression of survival data for secondary rearing study (Chapter 3).

	Log-Likelihood	χ^2	df	Pr>χ^2	R^2
Likelihood ratio test:	-990.9	1814.4	12	<0.001	
Wald test: Treatment		4624	11	<0.001	
Wald test: Day		1410.4	1	<0.001	
Goodness-of-Fit					
Hosmer & Lemeshow		0.9553	8	0.999	
McFadden					0.72
Cox & Snell					1.0
Nagelkerke					1.0

APPENDIX F
SIZE OF JUVENILE MUSSELS AT EACH SAMPLING PERIOD

Table F.1. Mean mussel size and standard deviations (µm) for experiment 1 yellow lampmussel juveniles (*Lampsilis cariosa*). Raw data averaged from initial replicates (n) per treatment. Value in parenthesis are number of individuals measured combined among replicates. Difference is difference in shell length between day 0 and day 53. Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$).

		Day									
Treatment	**n**	**0**	**4**	**11**	**18**	**25**	**32**	**39**	**46**	**53**	**Difference**
Algae Only (Control)	3	225 ± 3 (150)[b]	247 ± 5 (150)	234 ± 5 (150)	239 ± 1 (132)	252 ± 6 (54)	-	-	-	-	
Low	4	237 ± 9 (200)[a]	246 ± 6 (200)	270 ± 6 (200)	290 ± 2 (200)	321 ± 17 (200)	353 ± 13 (200)	394 ± 17 (200)	417 ± 23 (200)	448 ± 27 (200)	211 ± 37
Med	4	226 ± 2 (200)[b]	233 ± 6 (200)	291 ± 8 (200)	305 ± 7 (200)	329 ± 13 (200)	345 ± 12 (200)	373 ± 25 (200)	406 ± 35 (200)	426 ± 56 (200)	200 ± 66
High	4	229 ± 9 (200)[b]	220 ± 3 (200)	278 ± 5 (200)	296 ± 17 (200)	337 ± 10 (200)	360 ± 20 (200)	381 ± 28 (200)	408 ± 23 (200)	431 ± 34 (190)	202 ± 28
Probiotic Only (Control)	4	220 ± 8 (200)[b]	238 ± 2 (200)	260 ± 12 (200)	258 ± 5 (200)	266 ± 4 (200)	279 ± 9 (200)	284 ± 8 (48)	-	-	
Group Pr(>F)		<0.001									0.733

Table F.2. Mean mussel size and standard deviations (µm) for experiment 2 yellow lampmussel juveniles (*Lampsilis cariosa*). Raw data averaged from initial replicates (n) per treatment. Due to mortality and loss of complete replicates resulted in B1008 (n=1) sampling day 30; and NiteOut (n=3) sampling days 44 and 51. Value in parenthesis are number of individuals measured combined among replicates. Difference is difference in shell length between day 0 and day 51. Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$).

		Day								
Treatment	**n**	**0**	**7**	**16**	**23**	**30**	**37**	**44**	**51**	**Difference**
Algae Only (Control)	4	412 ± 9 (200)	450 ± 27 (200)	492 ± 17 (173)	515 ± 16 (60)	-	-	-	-	
B1000	4	423 ± 8 (200)	470 ± 12 (200)	481 ± 16 (200)	520 ± 16 (192)	565 ± 10 (184)	638 ± 38 (178)	689 ± 27 (176)	729 ± 11 (172)	306 ± 12
B1002	3	417 ± 8 (150)	455 ± 14 (150)	488 ± 16 (115)	-	-	-	-	-	
B1008	4	421 ± 8 (200)	494 ± 16 (48)	515 ± 23 (28)	505 ± 3 (15)	504 (4)	-	-	-	
N1100-50x	4	417 ± 15 (200)	462 ± 6 (200)	488 ± 13 (200)	538 ± 24 (75)	-	-	-	-	
NiteOut	4	421 ± 5 (200)	470 ± 11 (200)	522 ± 14 (200)	540 ± 16 (134)	571 ± 31 (107)	621 ± 10 (22)	664 ± 52 (31)	708 ± 97 (19)	285 ± 97
Glosso	4	406 ± 7 (200)	466 ± 7 (200)	506 ± 10 (156)	535 ± 8 (38)	-	-	-	-	
Group Pr(>F)		0.722								0.681

Table F.3. Mean mussel size and standard deviations (µm) for experiment 3 yellow lampmussel juveniles (*Lampsilis cariosa*). Raw data averaged from initial replicates (n) per treatment. Value in parenthesis are number of individuals measured combined among replicates. Difference is difference in shell length between day 0 and day 35. Means followed by a common letter are not significantly different (Tukey's post-hoc analysis, $p<0.05$).

		Day						
Treatment	**n**	**0**	**7**	**14**	**21**	**28**	**35**	**Difference**
LAC	3	285 ± 8 (150)^{b}	288 ± 7 (150)	362 ± 4 (150)	418 ± 15 (139)	491 ± 6 (144)	536 ± 8 (119)	251 ± 6^{a}
QAC	3	273 ± 4 (150)^{b}	302 ± 9 (150)	354 ± 2 (147)	428 ± 41 (105)	423 ± 22 (119)	472 ± 20 (108)	200 ± 23^{a}
LNA	4	300 ± 11 (200)^{b}	325 ± 12 (200)	356 ± 18 (200)	381 ± 8 (186)	393 ± 9 (165)	393 ± 24 (124)	92 ± 22^{c}
LBA	3	253 ± 7 (150)^{ab}	248 ± 16 (125)	298 ± 9 (40)	349 ± 27 (21)	434 ± 31 (15)	432 ± 37 (9)	179 ± 15^{b}
QNA	3	323 ± 16 (150)^{b}	338 ± 6 (150)	380 ± 23 (150)	428 ± 31 (150)	419 ± 22 (150)	448 ± 9 (128)	125 ± 8^{bc}
QBA	3	231 ± 9 (150)^{a}	256 ± 5 (147)	290 ± 8 (70)	336 ± 26 (18)	374 ± 23 (17)	413 ± 23 (29)	182 ± 20^{b}
QN	2	233 ± 3 (100)^{a}	222 ± 0.2 (90)	229 ± 1 (19)	-	-	-	
LBNA	4	249 ± 5 (200)^{ab}	249 ± 10 (112)	274 ± 20 (41)	-	-	-	
Group Pr(>F)		<0.001						<0.001

Table F.4. Juvenile mussel size (mean ± standard deviations (mm)) for Chapter 3 eastern lampmussel (*Lampsilis radiata*). Raw data averaged from initial replicates per treatment (n). 25 mussels were measured per replicate, except for the Trough-1 (small) NANFH system where values in parenthesis are number of individuals measured combined among replicates.

			Day					
Treatment	**Location**	**n**	**0**	**14**	**28**	**44**	**49**	**56**
Dogpan	CARC	3	5.60 ± 0.17	6.58 ± 0.13	7.70 ± 0.07	n.d.	8.37 ± 0.37	8.69 ± 0.12
Airlift Upweller	CARC	3	5.18 ± 0.70	5.55 ± 0.14	8.25 ± 0.32	n.d.	10.55 ± 0.40	10.38 ± 0.54
Tank Upweller	CARC	4	5.48 ± 0.10	6.28 ± 0.18	9.58 ± 0.65	n.d.	10.2 ± 0.18	10.00 ± 0.51
Trough-1 (small)	CARC	3	3.98 ± 0.07	4.28 ± 0.06	5.27 ± 0.06	n.d.	6.17 ± 0.34	6.25 ± 0.34
Trough-2 (large)	CARC	3	5.69 ± 0.11	5.56 ± 0.21	7.84 ± 0.29	n.d.	9.81 ± 0.12	9.90 ± 0.70
Dogpan	NANFH	3	5.46 ± 0.21	5.61 ± 0.13	5.96 ± 0.04	5.88 ± 0.16	n.d.	5.83 ± 0.14
Airlift Upweller	NANFH	2	5.47 ± 0.08	6.03 ± 0.15	6.82 ± 0.09	6.73 ± 0.21	n.d.	7.06 ± 0.04
Trough-1 (small)	NANFH	3	3.98 ± 0.13	4.21 ± 0.04	4.53 ± 0.08	4.52 ± 0.13 (61)	n.d.	4.81 ± 0.09 (36)
Trough-2 (large)	NANFH	3	5.47 ± 0.02	5.89 ± 0.19	5.91 ± 0.28	5.75 ± 0.11	n.d.	6.00 ± 0.05
Baskets-Pond	NANFH	3	5.61 ± 0.12	7.05 ± 0.17	10.78 ± 0.83	12.44 ± 0.87	n.d.	14.24 ± 0.39
Baskets-Hatchery	NANFH	3	5.34 ± 0.11	5.40 ± 0.19	5.63 ± 0.15	5.58 ± 0.27	n.d.	5.65 ± 0.09

APPENDIX G
WATER QUALITY FIGURES FOR EXPERIMENT 3 (CHAPTER 2)

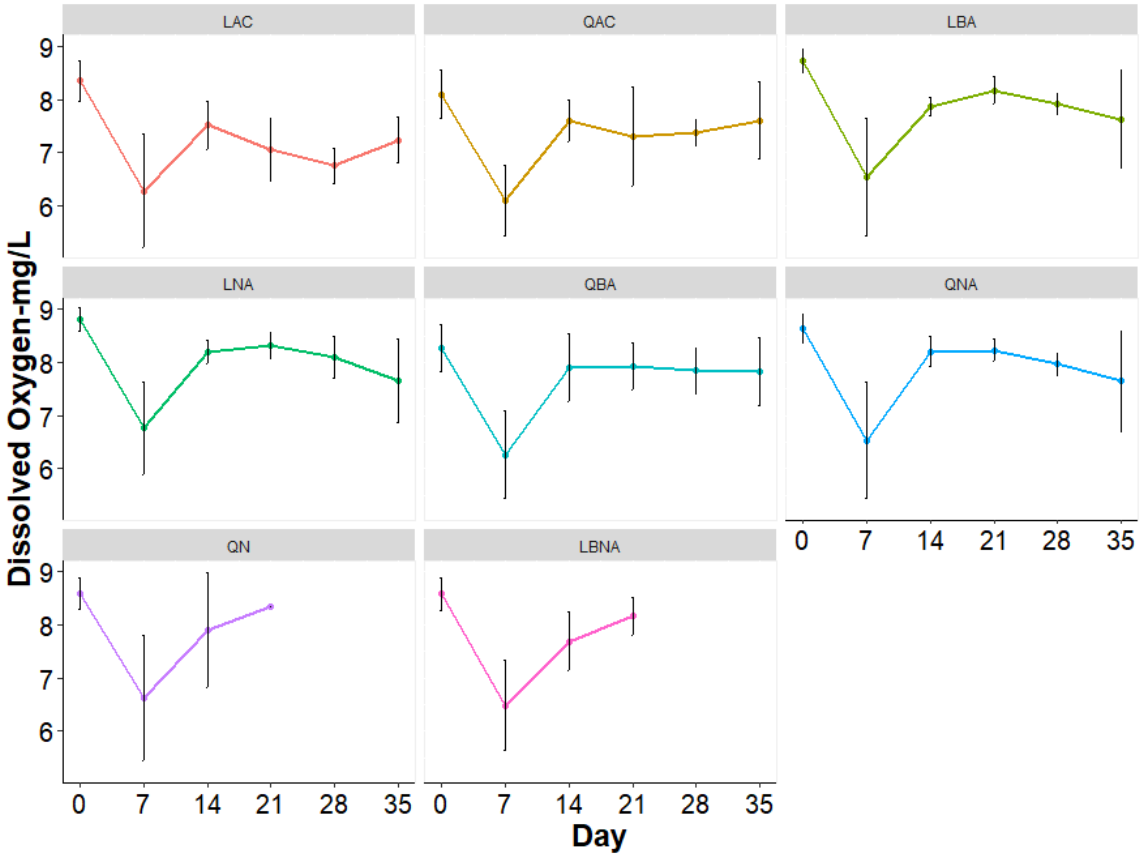

Figure G.1. Observed average dissolved oxygen (DO) (mean ± SD) for experiment 3 each week per treatment. Average DO is plotted on the first day of each week (e.g. average DO of days 0-7 are plotted on day 0).

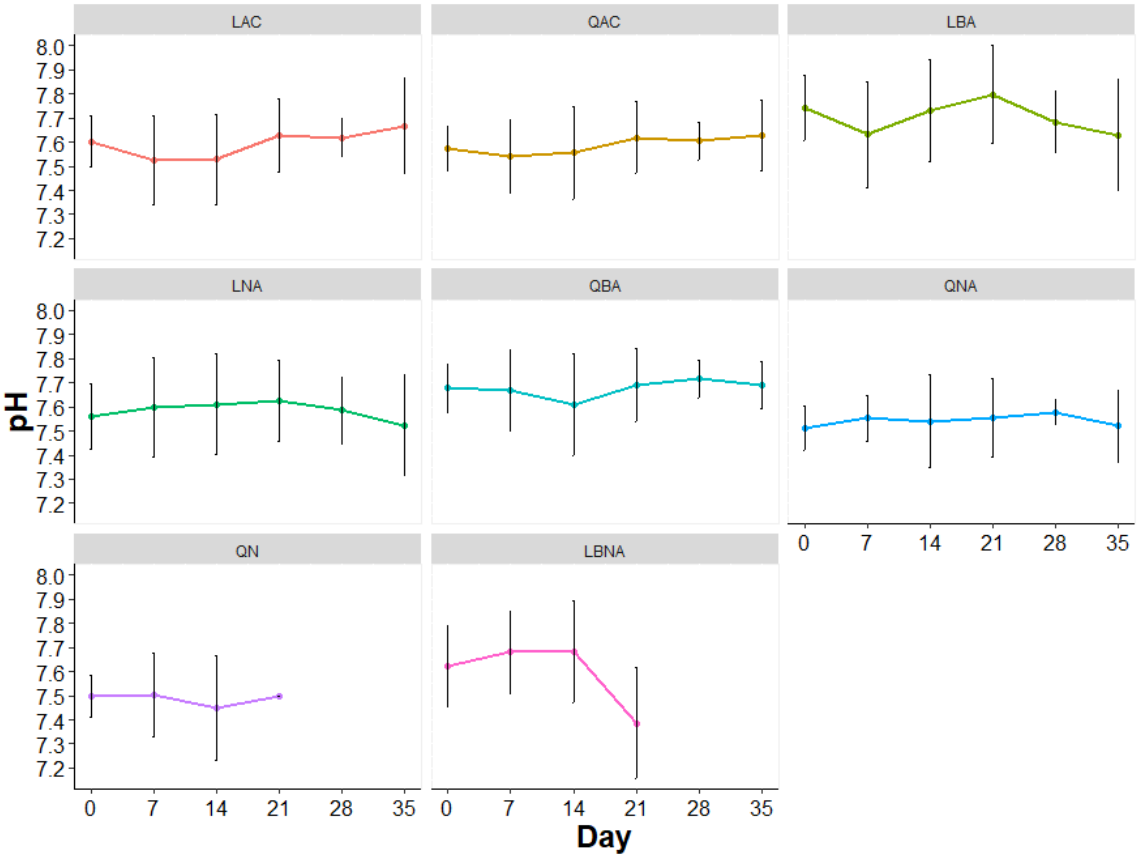

Figure G.2. Observed average pH (mean ± SD) for Experiment 3 each week per treatment. Average pH is plotted on the first day of each week (e.g. average pH of days 0-7 are plotted on day 0).

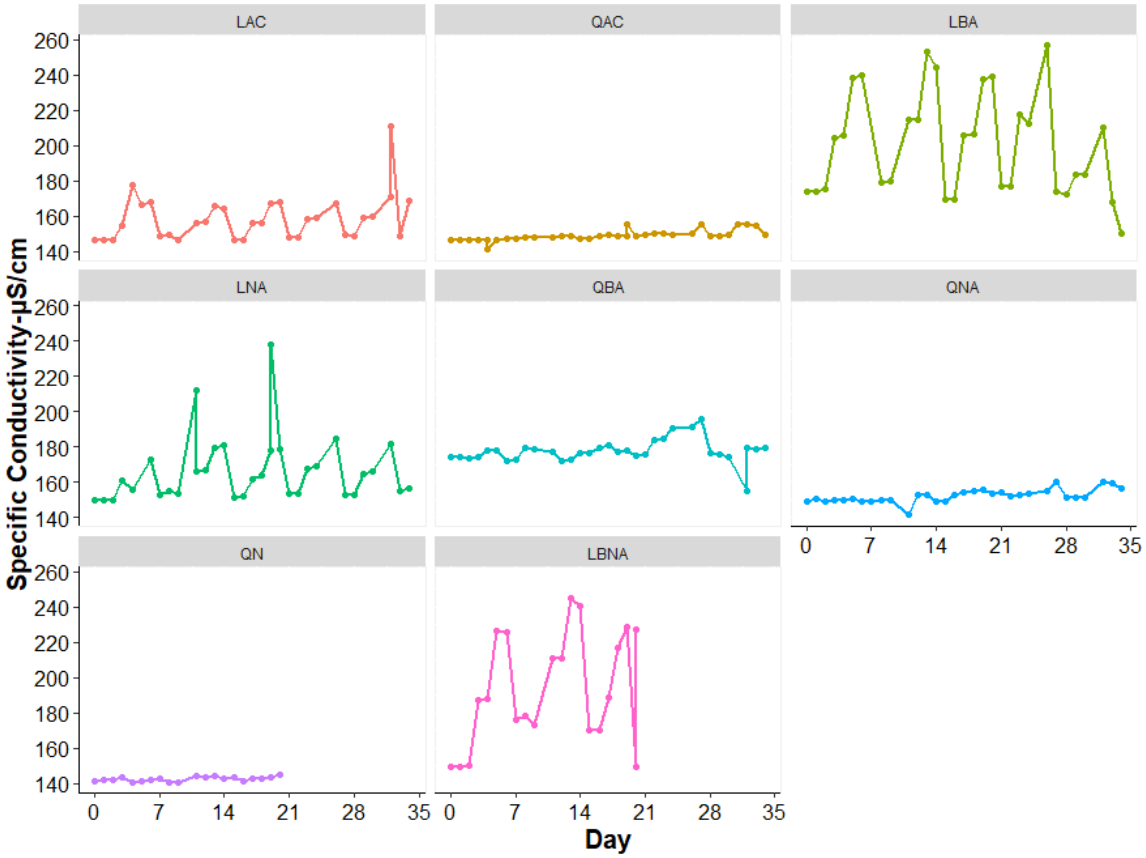

Figure G.3. Observed specific conductivity for Experiment 3 each day per treatment. Each dot represents a conductivity measurement on each day.

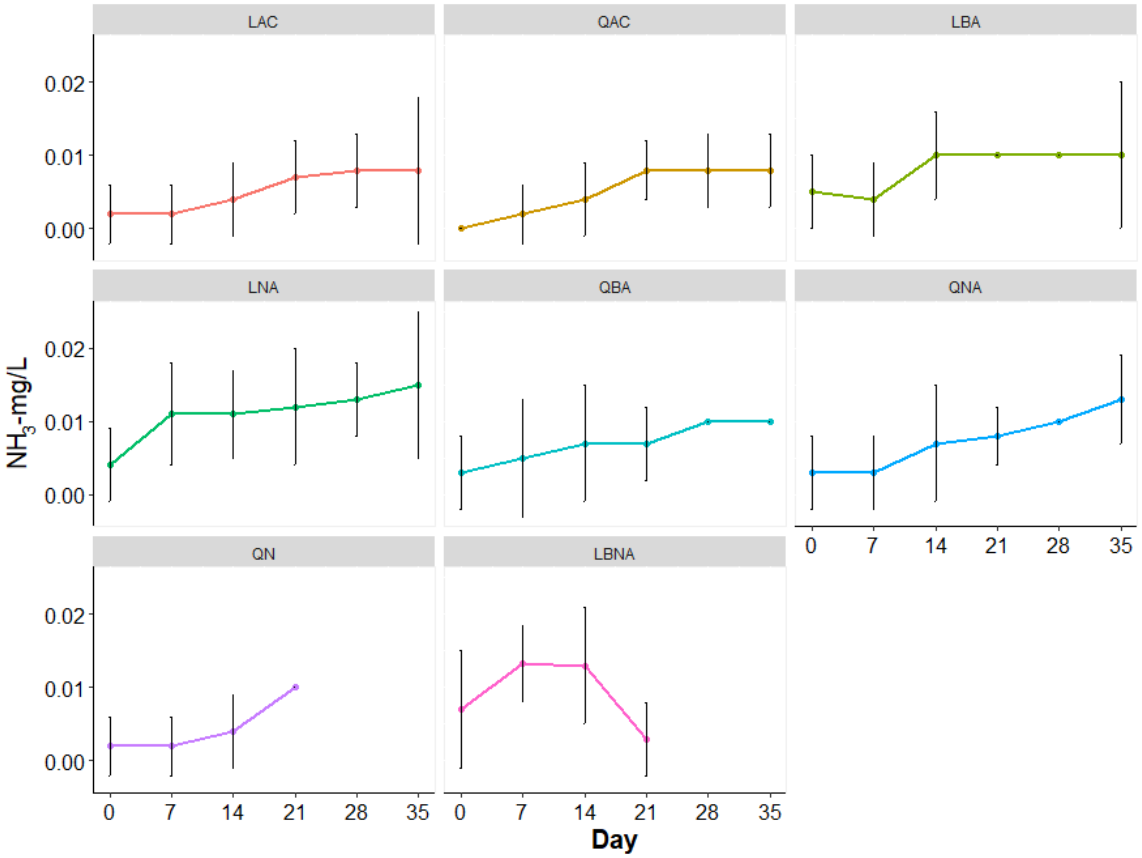

Figure G.4. Observed average NH_3 (mean ± SD) for Experiment 3 each week per treatment. Average NH_3 is plotted on the first day of each week (e.g. average NH_3 of days 0-7 are plotted on day 0).

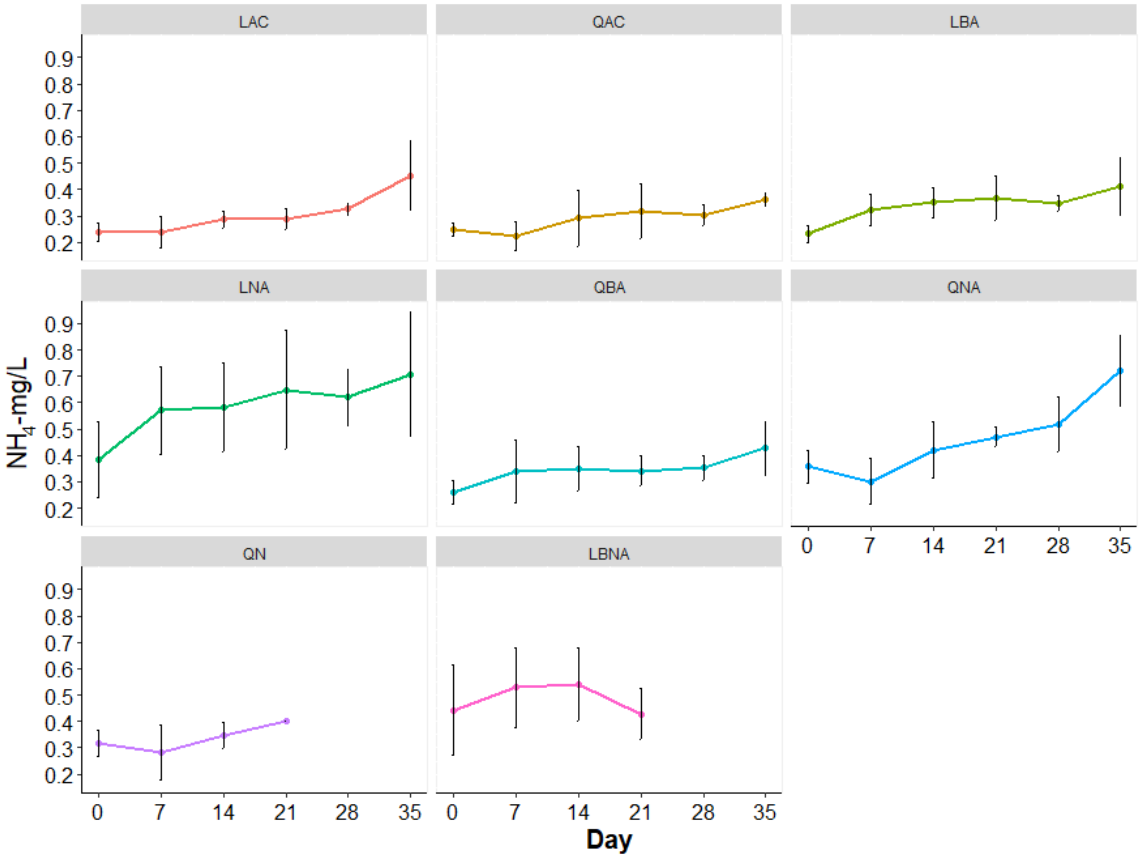

Figure G.5. Observed average NH_4 (mean ± SD) for Experiment 3 each week per treatment. Average NH_4 is plotted on the firt day of each week (e.g. average NH_4 of days 0-7 are plotted on day 0).

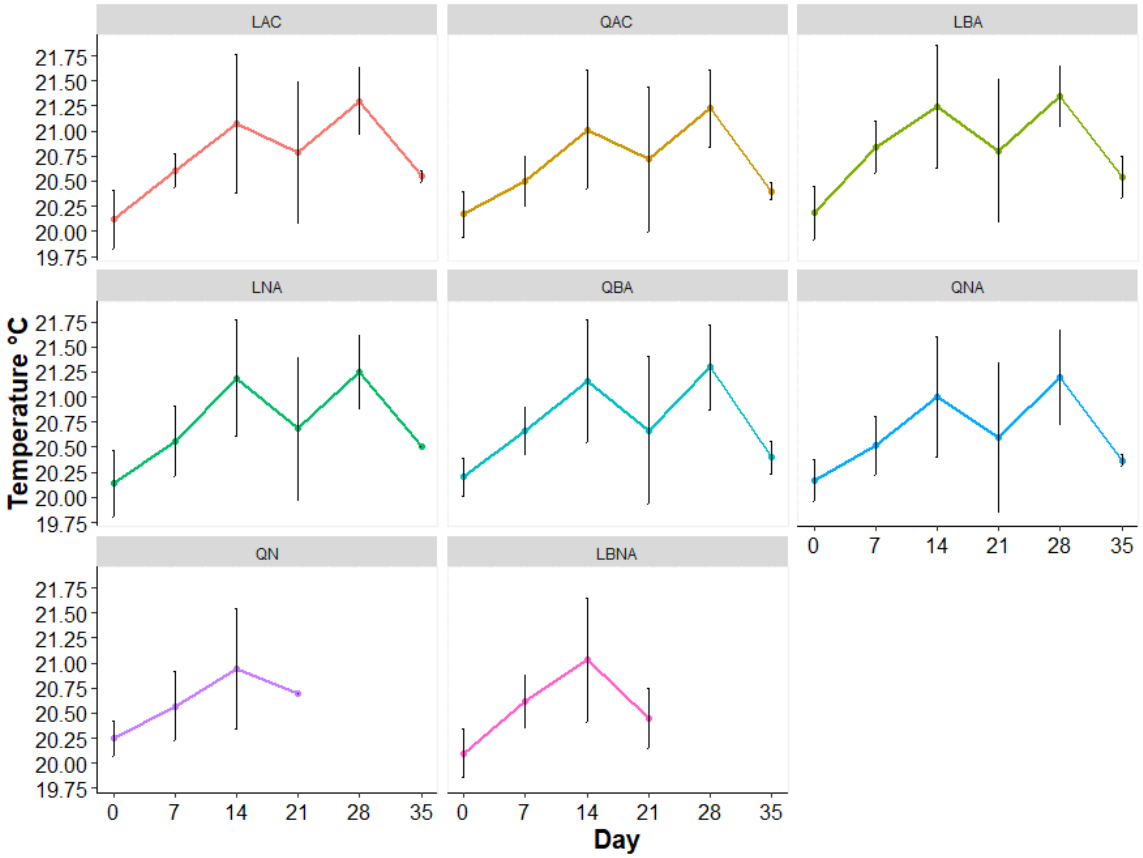

Figure G.6. Observed average temperature (mean ± SD) each week per treatment for Experiment 3. Average temperatures are plotted on the first day of each week (e.g. average temperature of days 0-7 are plotted on day 0).

APPENDIX H
WATER QUALITY COMPARISON BETWEEN PAIRED REARING SYSTEMS AT CARC AND NANFH (CHAPTER 3)

Table H.1. Water quality analysis for comparison of CARC and NANFH treatments (mean ± SD) using Welch's two sample t-test for unequal variance. Bold indicates significant group differences ($p<0.05$) and an asterisk (*) indicates different sample sizes for analysis.

	Water Quality Variable								
Rearing System	**Temperature (°C)**			**Dissolved Oxygen (mg/L)**			**pH**		
	CARC	**NANFH**	**p-value**	**CARC**	**NANFH**	**p-value**	**CARC**	**NANFH**	**p-value**
Dogpan	25.5 ± 1.1	20.3 ± 1.8	**<0.001**	8.27 ± 0.42	8.65 ± 0.27	**0.007**	7.40 ± 0.18	7.17 ± 0.07	**<0.001**
Airlift Upweller	19.9 ± 2.3	19.6 ± 1.8	0.263	7.67 ± 0.45	8.38 ± 0.58	**0.006**	7.24 ± 0.08	7.09 ± 0.04	**<0.001**
Trough	19.2 ± 2.2	19.9 ± 1.8	0.124	7.98 ± 0.44	7.55 ± 0.87	0.108	7.30 ± 0.24	7.26 ± 0.10	0.573
	TDS (ppm)			**Salinity (ppm)**			**Specific conductivity (µS/cm)**		
Dogpan	102 ± 4	362 ± 36	**<0.001**	72 ± 4	243 ± 27	**<0.001**	156 ± 9	537 ± 52	**<0.001**
Airlift Upweller	58 ± 8	386 ± 46	**<0.001**	40 ± 0	256 ± 31	**<0.001**	100 ± 3	555 ± 64	**<0.001**
Trough	56 ± 11	374 ± 36	**<0.001**	40 ± 0	254 ± 27	**<0.001**	102 ± 3	549 ± 53	**<0.001**
	Ammonium (NH_4-N mg/L)			**Calcium Carbonate ($CaCO_3$) (mg/L)**			**Chloride (Cl-) (mg/L)**		
Dogpan	0.10 ± 0.06	0.09 ± 0.09	0.724	15.8 ± 7.4	39.8 ± 6.9	**<0.001**	3.3 ± 2.6	3.58 ± 1.35	0.735
Airlift Upweller	0.05 ± 0.04	0.04 ± 0.03	0.601	16.8 ± 5.8	38 ± 8.8	**<0.001**	5.6 ± 2.0	1.20 ± 1.39	**<0.001**
Trough	0.07 ± 0.09	0.02 ± 0.01	0.061	13.4 ± 4.2	40.4 ± 16.6	**<0.001**	4.3 ± 3.3	2.00 ± 2.29	**0.039**

APPENDIX I
AVERAGE TEMPERATURE PER SAMPLING INTERVAL AT CARC AND NANFH (CHAPTER 3)

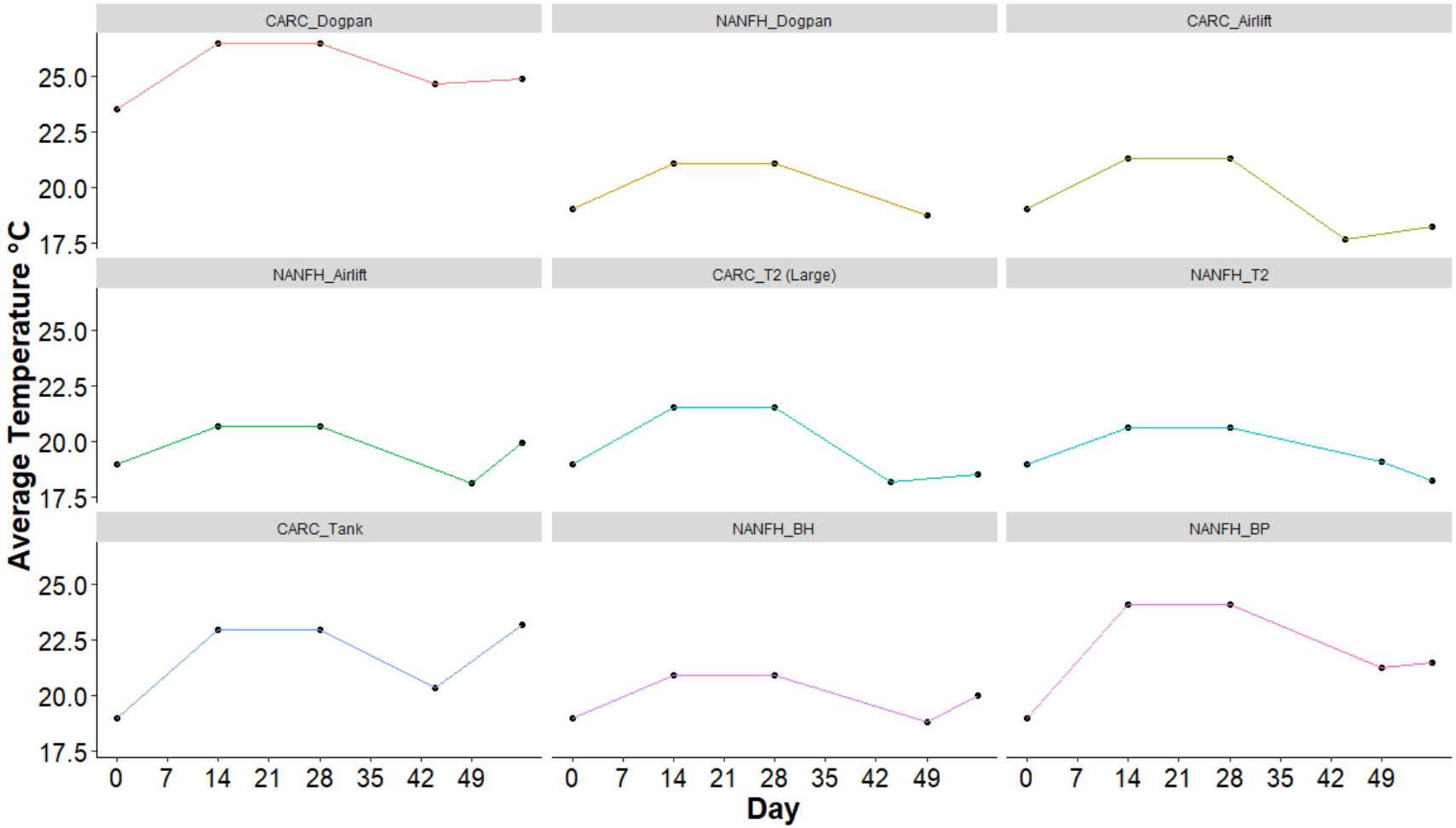

Figure I.1. Observed average temperature (mean ± SD) each sampling period per rearing system (Chapter 3). Average temperatures are plotted on the last day of each week (e.g. average temperature of days 0-7 are plotted on day 7).

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